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Editor
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THE EDITOR'S VIEWPOINT

Berggren and Hedström (p. 161) found that the addition of sugar solutions to tetanus toxin increased the penetration of the toxin through enamel. However, sugar solutions did not increase the penetration of dyes under the condition used by the authors. Since sugar solutions are often ingested with various drugs, as well as being parts of the dietary, the importance of the changes in the permeability of enamel under these conditions is quite significant.

Berggren and Hedström (p. 169) report that the permeability of enamel to a toxin-fructose mixture was not changed by treating the teeth with fluorine. It has been established that applying fluorides to teeth increases their hardness and decreases their susceptibility to caries. That the enamel is still permeable despite the application of fluorine solutions suggests the need of further investigation.

McClure and Likins (p. 172) found approximately 30 per cent increase in the fluorine content of dentin of teeth exposed to 1.0 to 1.2 p.p.m. of fluorides in the drinking water from birth as compared with teeth from individuals from nonfluoride regions. They believe that this indicates that the deposition of fluorides in teeth may be an important factor in caries reduction in individuals from optimal water fluoride regions. More information is needed before the mechanism of fluorides as caries inhibitors is established. At such time, a rational approach may be substituted for the empirical methods now in use, or the present technics may be shown to be best on a scientific basis.

Suess and Fosdick (p. 177), using powdered enamel soaked in 2 per cent aqueous solution of sodium fluoride for three hours, found that the fluoridized enamel started to decalcify at about 0.5 pH units lower than untreated enamel. From this finding they postulate that fluoridized anterior teeth are more effectively protected than posterior teeth and that topical application of fluorides should give almost complete protection in instances of mild caries activity but in individuals with rampant caries it would effect only a delaying action. It is unfortunate that laboratory studies such as these are not designed to simulate clinical use more closely by applying the fluoride solution as recommended, i.e., four applications of approximately five minutes each. The artificial condition of powdered enamel, with its great surface areas, should also be avoided where feasible.

Smith and Gardner (p. 182), with the collaboration of Voss, Wing, Dunn, Figueras, and Keating, have developed a technique for determination of fluoride in blood. With increasing prophylactic application of fluorides, to water supplies, dentifrices, etc., and increased commercial use of fluorides, the possibility of toxicity must be considered. Without being an alarmist, possible cumulative and multiple application effects are not to be overlooked. The method described may be valuable to only a few investigators but to them it is of extreme importance.

Palmer, Overstreet, and Sacks' (p. 189) study of the effects of acidulated fluoride, aqueous fluoride, and Zin-Ferro-Nitrate supplies data for speculation. If we assume that weighing of enamel fragments is an accurate enough method for differentiation at the levels observed, then it can be accepted that acidulated fluoride, sodium fluoride not acidulated, and Zin-Ferro-Nitrate are effective in reducing decalcification (in vitro) in the order listed. However, clinical studies of Roberts, Bibby, and Wellock (*J. D. Res.* **27**: 497, 1948) indicate that acidulated fluorides are ineffective in vivo; studies of Ast, Bushel, and Chase (*J.A.D.A.* **41**: 437, 1950) and of Pelton (*J. D. Res.* **29**: 756, 1950) show the relative ineffectiveness of Zin-Ferro-Nitrate, while numerous reports give a high value to aqueous sodium fluoride. It might be conjectured that the differences between the clinical results and the laboratory results indicate that none of the solutions produces its major effect through reducing the solubility of enamel. Suess and Fosdick (p. 177) offer another explanation.

Using strictly in vitro methods, Palmer, Overstreet, and Sacks (p. 195) have demonstrated that acidulated or nonacidulated fluoride solutions and Zin-Ferro-Nitrate solution become progressively less effective in reducing decalcification as enamel and dentin are exposed for longer periods to acids. Whether it can be assumed, on the basis of the evidence, that the effectiveness of topically applied fluorides is gradually lost is open to question. Many in vitro results on enamel fragments have not been substantiated by chemical studies. A second conclusion of the authors, i.e., that fluoride solutions are as effective when applied to partially decalcified tooth fragments as when applied to intact tooth fragments, raises the question of whether topically applied fluorides do stop incipient caries. Whether any of our methods used in clinical studies of caries prophylaxis measures the inhibition of caries initiation, or even of incipient caries, can be debated.

In a further study, Palmer, Overstreet, and Sacks found acidulated fluorides more effective than nonacidulated fluorides in reducing solubility of enamel in acid (culture of lactobacilli). This phenomenon, which is contrary to clinical results, is intriguing. As suggested by the authors, variations in the technique of applying fluorides may be worthy of more intensive and extensive study. Would it be of clinical value to use some device, such as that employed experimentally by Adler, Straub, and Popovics (*J. D. Res.* **29**: 549, 1950), to keep teeth under the influence of fluorides for a prolonged period?

Lundqvist (p. 203) demonstrated that while iodoacetic acid given in food is an inhibitor of glycolysis and of dental caries, its toxicity is too great to justify its use for caries control. Lundqvist's experimental method is offered as a basis for studies of other inhibitors of glycolysis which may prove less toxic and thereby applicable for caries prophylaxis.

Malcolm, Deaker, and Bell (p. 214) found no evidence that cetylamine would be toxic if used in a dentifrice under conditions of clinical use. This substance had been suggested by Walsh and Green (*J. D. Res.* **29**: 270, 1950) as a possible anticaries agent for use in a dentifrice. This is a portion of the background work essential before a substance suggested for clinical use can be evaluated adequately.

Green and Walsh (p. 218) have extended their studies on the protective action of amines in mineral oil on enamel surfaces. It is obvious that reducing the solubility of enamel offers some hope of protection against caries. Most efforts have been directed at changing the enamel composition but Green and Walsh propose to supply a protective layer over the enamel surface. Clinical trial of their method appears to be justified on the basis of their in vitro findings.

Mavrogordato (p. 225) reports that the caries rate is very low in a Greek community with a high fluoride content (3 p.p.m.) in its domestic water supply. He points out that the deciduous teeth of the children in this town are not mottled, as are the permanent teeth, but that the deciduous teeth do not resist dental caries. This suggests that not more than 1.5 p.p.m. of fluorides pass the placental barrier. The teeth of young individuals who moved into the community after dental calcification show some reduction in caries activity. The latter observation is more in line with the results of preliminary studies in communities with artificially fluoridated waters than with previous data from naturally fluoridated waters.

From the study of the observation of glucose degradation products in the presence of saliva, Calandra and Adams (p. 229) have shown there is a rapid oxidation of lactate and slight oxidations of pyruvate, acetate, and propionate in the presence of saliva. The authors believe that enzyme systems capable of causing a rapid oxidation of lactic acid offer the most promising explanation of natural caries immunity. They believe that such enzymes are contributed by the bacterial flora of the mouth. These chemical studies should be considered in relationship with the findings of bacteriologists in studies on the flora of the dentobacterial plaque and the mouth as a whole. While it may be possible to implant favorable types of organisms in the oral cavity, there may be means of altering the components of the present flora so that the activity of certain enzyme systems are emphasized over those of others.

Nevin and Walsh (p. 235) investigated the possible effects of certain physicochemical factors in relationship to the cause of caries.

Basic studies in this field are long overdue. Using specially prepared glass rods arranged in an apparatus to measure pH changes, Nevin and Walsh obtained results which can explain differences in caries susceptibility of different interproximal surfaces of the same tooth and between interproximal surfaces in different parts of the mouth. The indications are that variations in degree of separation of teeth (of even minor degree) and the shape and width of contacts are more important than buffering capacity of saliva.

Kitchin, Robinson, Permar, and Imhoff (p. 251) have studied a series of salivary lactobacillus counts from patients before and after the topical application of 2 per cent sodium fluoride solution. Their findings confirm the previously reported observation that salivary lactobacillus counts show no consistent difference after topical treatment with 2 per cent sodium fluoride. It is proposed that the reduction in dental caries which is reported after the topical application of 2 per cent sodium fluoride and the failure of lactobacillus counts to be reduced are not inconsistent. The reduction in caries which has been reported (40 per cent) is not a cessation of caries activity, yet a reduction of lactobacillus counts to *zero* or near zero would indicate practically a complete stoppage of the carious process.

Montgomery and von Haam (p. 260) found no striking cell type that they could consider as characteristic of leucoplakia in smears studied by the Papanicolaou technic. They used white, leathery, keratinized patches, seen clinically, as diagnostic for leucoplakia. Only increased keratinization was observed in their study of the exfoliative cytology. Whether other changes could have been observed in surface cells from areas of dyskeratosis is problematical.

Butcher and Taylor (p. 265), by experimental studies on monkeys, have clearly demonstrated that teeth develop and maintain normal structure without a nerve supply. Even interference with vascularity, except directly at the apex, seldom caused permanent injury. Surprisingly, the pulps of teeth with large apical foramina were more easily injured than those of teeth with small apical foramina. Regeneration of injured pulp tissue was observed. Repeated observations of the regenerative powers of the pulp and the availability of effective antibiotics, which are noninjurious to tissues, should lead to more conservative pulp therapy and dispel the older concept of the extreme inability of the pulp to recuperate following injury.

The relative importance of genetic factors and function on bone development has attracted attention for many years. Removing the temporalis muscle of young rats and studying changes in the mandibles by grenz ray, Horowitz and Shapiro (p. 276) have demonstrated that functional changes affect both the external configuration and the internal architecture. Most striking is the absence of the coronoid processes on the operated sides of the experimental animals.

Since agar-agar impressions are usually immersed in 2 per cent solution of potassium sulfate to control volumetric change, Luster (p. 281) studied the effect of this solution and other sulfates on agar gels. She found that potassium sulfate failed to control shrinkage. Some of the other sulfates were better but Luster warns against immediate transfer of the experimental results to dental applications. The suggestion that each brand of hydrocolloid requires its own solution for control of dimensional change appears to be sound.

Taylor and Peyton (p. 290) compared properties of gold wires used in orthodontics and partial denture prosthesis under tension and bending. The results with treated and combined treated and aged conditions are given. Investigation of the properties of materials used in dentistry should improve opportunities for clinicians.

At a recent meeting of the Board of Editors, questions were raised as to the feasibility of continuing The Editor's Viewpoint. After some discussion, those members of the Board of Editors present voted unanimously to continue the publication of The Editor's Viewpoint but it was suggested that a short word of explanation be inserted concerning the background of this page.

The Editor's Viewpoint was first published in the February, 1946, issue of the JOURNAL of DENTAL RESEARCH (*J. D. Res.* **25**: 16, 1946). At that time, the following statement appeared: "The Editor wishes to experiment with a page of comments. If the readers indicate a desire for it to be con-

tinued such a policy will be discussed with our Board of Editors. Until such a time, we can call this an editorial research problem.'' The enthusiastic response from some of our readers led the Editor to continue with the experiment and the desirability of the page has been discussed with the Board of Editors from time to time.

The Editor's Viewpoint is not intended as a summary of the articles published in the JOURNAL for such would be a repetition of the conclusions which are usually published with each paper.

Neither is The Editor's Viewpoint intended as a critical evaluation of the papers from a technical standpoint. The Editor's Viewpoint simply gives the Editor's own personal reaction to the paper, suggesting possible clinical applications in some instances, and in others pointing out the need for further investigation. The Editor's Viewpoint will probably be of little use to scientists in their own fields except to stimulate thought toward different goals. We hope that there are increasing numbers of readers of the JOURNAL of DENTAL RESEARCH who are not actively engaged in dental research who may apply the results of dental research in practice. For them, we hope The Editor's Viewpoint serves a useful function. It is simply, as stated, one man's viewpoint.

H. B. G. R.

EXPERIMENTAL STUDIES IN VIVO ON THE PERMEABILITY OF ENAMEL WITH PARTICULAR REGARD TO THE EFFECT OF SUGAR SOLUTIONS

HELGE BERGGREN, ODONT.D., AND HARRY HEDSTRÖM, VET.M.D.

Department of Operative Dentistry, State Dental College and State Veterinary Medical Institute, Stockholm, Sweden

PREVIOUSLY one of us investigated the permeability of enamel in vivo to radioactive isotopes, dyes, bacteria, and bacterial toxin. (Berggren, 1947.*) In experiments on human beings it was shown that methylene blue penetrated and stained limited areas of the enamel, entering this from the dentin. The stained structures were studied by means of photomicrographs.

By the use of radioactive phosphorus in experiments on human beings and dogs it was shown that enamel is permeable to phosphorus atoms in both centrifugal and centripetal directions. Where radioactive sodium was used the quantity taken up was very large in comparison with the amount of sodium usually present in the enamel, a fact which suggests an ion exchange. As sodium is amply present in the intercellular fluid, the large quantity taken up indicates the possibility of a fluid circulation in the enamel.

No permeability of the enamel to bacteria could be established. The enamel was found to be centripetally permeable to tetanus toxin in the presence of glucose, but not when the toxin was applied alone. All the experiments with bacteria and bacterial toxin were carried out on dogs.

In connection with the theory of dental caries as an inflammatory disease, it was of the greatest interest to establish whether tetanus toxin is capable of penetrating enamel in amounts sufficiently large to be detected by biologic test only when mixed with a sugar solution.

Several dental research workers consider sugar a potent factor in promoting caries activity. It was therefore desirable to verify these interesting results in a larger number of experiments. Such a series was carried out in order to throw light on the following problems: (1) The relation between glucose concentration and toxin penetration. (2) The influence of fructose upon the toxin penetration. (3) The influence of different sugar solutions upon the penetration of dyes.

1. The Relation Between Glucose Concentration and Toxin Penetration

MATERIAL AND METHODS

The experiments were carried out on 20 dogs 6 to 12 months of age.

Experimental Technic.—The experimental technic suggested by Berggren was applied.

Received for publication, Oct. 6, 1950.

*Berggren, H.: Experimental Studies on the Permeability of Enamel and Dentine, *Svensk Tandl.-Tidskrift (J. Swedish D. A.)* **40**: No. 1 B, 1947.

The canines were fitted with copper bands fastened with cement joints at the cervix, the entire crown being left free from cement. The copper bands were then filled with a mixture consisting of equal quantities of (a) tetanus toxin in broth, having a titer of 1:20,000 and 1,200,000, and (b) a glucose solution 2 per cent to 50 per cent. The mixture has thus a titer of 1:10,000 and 1:100,000, and a glucose concentration of 1 per cent to 25 per cent (Tables I and II). Over the opening of the copper bands, "roofs" of wax were applied, the edges being well sealed. The animals were kept under morphine for periods ranging from 2 to 41 hours, at the end of which they were sacrificed. The copper bands were removed and the teeth thoroughly washed in running water before sectioning and removing the pulps.

Test Method.—The pulp tissue was ground up in a mortar with sterile sand after addition of a little saline. After being pressed through a copper wire of 250 mesh a suspension was prepared from it.

The toxicity of the pulp suspension was tested by intramuscular injection into mice. Doses of the suspension as large as 0.5 ml. were injected into the muscles of the hind leg. Of four mice so treated, two were first immunized by a dose of tetanus antitoxin (0.2 ml. containing 620 International units per milliliter) to act as controls. In those cases where the nonimmunized mice died or developed symptoms of tetanus, and the serum-protected were symptom free, it was considered as evidence of penetration of the toxin through the dental hard tissues.

TABLE I

RELATION BETWEEN GLUCOSE CONCENTRATION AND TOXIN PENETRATION; "FOUR-TEETH" EXPERIMENTS

NO.	TIME OF ACTION (HR.)	TOXIN TITER	GLUCOSE CONC. (%)	PULP VOLUME	RESULTS
1	28	1:100,000	20	Large	+
2	28	1:100,000	25	Small	–
3	2½	1:100,000	25	Large	–
4	2½	1:100,000	25	Large	–
5	29	1:100,000	5	Small	–
6	29	1:100,000	5	Small	–
7	41	1:100,000	5	Large	–
8	3	1:100,000	5	Small	–
9	15	1:100,000	2	Large	+
10	29	1:100,000	1	Large	–
11	28	1:100,000	1	Small	–
12	28	1: 10,000	25	Large	–
13	25	1: 10,000	25	Large	–
14	27	1: 10,000	5	Large	–

In the first series of 14 animals (Table I) the pulps from all four canines of the same dog were ground together, and the penetrative effect was compared in *different* animals. This, however, was not the case in the subsequent series (Table II). In this series the comparisons were made between *homologous teeth in the same animal*, in order to eliminate as far as possible the uncertainty that is always associated with biologic research work in vivo when using this experimental technic. Thus the copper bands around the right upper and

lower canines were filled with toxin in a 20 per cent glucose solution, whereas on the left side a 10 per cent glucose solution was used. In this way the experiments were performed using the pulp tissue from only two teeth, and an amount of pulp tissue only half as large as in the first series was obtained. Consequently the amount of toxin inoculated into the mice as a test was only half as large, and it was by no means certain that it would be sufficient to give a positive result.

The terms "four-teeth" and "two-teeth" experiments are used in the following text to indicate first and second series respectively. In the tables a minus sign indicates a negative result, i.e., no inoculated mice showed symptoms of tetanus. A plus sign indicates that, of the mice inoculated, those that were immunized were symptom free and the mice *not* immunized died from tetanus. (+) indicates that the immunized mice were symptom free and those *not* immunized had the typical symptoms of tetanus but survived.

TABLE II

RELATION BETWEEN GLUCOSE CONCENTRATION AND TOXIN PENETRATION; "TWO-TEETH" EXPERIMENTS

NO.	TIME OF ACTION (HR.)	TOXIN TITER	GLUCOSE CONC. (%)		PULP VOLUME	RESULTS	
			LEFT CANINES	RIGHT CANINES		10% GLUCOSE CONC.	20% GLUCOSE CONC.
15	31	1:100,000	10	20	Large	–	+
16	29	1:100,000	10	20	Large	–	+
17	28	1:100,000	10	20	Small	–	–
18	30	1:100,000	10	20	Large	–	+
19	24	1:100,000	10	20	Large	–	+
20	33	1:100,000	10	20	Large	–	+

RESULTS

In the experiments by Berggren positive results were obtained with a mixture consisting of equal parts of a tetanus toxin with the titer 1:200,000 and a glucose concentration of at least 40 per cent, i.e., the toxin titer of the mixture was 1:100,000 and the glucose concentration at least 20 per cent.

As it appears from Table I, a toxin penetration was now established at a high glucose concentration in one case (No. 1). As in previous experiments giving positive results, there were in this, too, other favorable conditions, i.e., a long time of action, a high toxin titer, and a great pulp volume.

Three experiments with high glucose concentration and toxin titer—two (Nos. 3 and 4) with a short time of action and one (No. 2) with a small pulp volume—were negative, as were two experiments (Nos. 12 and 13) where the toxin titer was low.

Among the experiments performed with low glucose concentration (1 per cent to 5 per cent) favorable conditions were present in only three instances (Nos. 7, 9, and 10). A positive result was obtained in case No. 9 with a glucose concentration of only 2 per cent. A toxin penetration at so low a glucose concentration is remarkable, and we are tempted to conclude that a penetration is possible under certain favorable conditions even without the addition of glucose.

Of the six "two-teeth" experiments, the results of which are given in Table II, one was totally negative on account of the small amount of pulp tissue while for the other five the inoculation was positive with pulp suspension from the teeth affected by toxin in 20 per cent glucose. On the other hand, a negative result was obtained from the teeth on the opposite side of the jaw and a 10 per cent concentration of glucose.

However, it cannot be stated that there will be no penetration at all of toxin in a 10 per cent glucose solution. It might very well have occurred in an amount too small to be detected. Perhaps a positive result would have been obtained if "four-teeth" experiments had been carried out, and twice the amount of pulp tissue had been available.

The results of these experiments indicate that there is a definite relationship between the concentration of the glucose solution and the penetrative capacity of the toxin so that the penetration increases with glucose concentration.

2. The Influence of Fructose Upon the Toxin Penetration

There is conclusive evidence that the passage of tetanus toxin through enamel is positively affected by the addition of a glucose solution, and that there exists a relationship between glucose concentration and toxin penetration.

For further investigations in this field experiments with fructose, the other component of common sugar, were also carried out.

Material and Methods

For these experiments 12 dogs were used. The above-described experimental technic and test methods were applied, and the investigation was arranged as "two-teeth" experiments. The penetrative capacity of the toxin was studied by comparing toxin-glucose on the right side of the jaws with toxin-fructose on the left side. (Table III.)

Table III

Influence of Fructose on Toxin Penetration; "Two-Teeth" Experiments

No.	Time of Action (Hr.)	Toxin Titer	Glucose Conc. Right Canines (%)	Fructose Conc. Left Canines (%)	Pulp Volume	Results	
						Glucose	Fructose
1	44	1:100,000	20	20	Small	(+)	+
2	43	1:100,000	20	20	Large	+	+
3	41	1:100,000	20	20	Small	–	–
4	38	1:100,000	20	20	Small	–	–
5	45	1: 10,000	20	20	Large	–	+
6	17	1: 10,000	20	20	Large	–	+
7	43	1: 10,000	20	20	Large	–	+
8	42	1: 10,000	20	20	Large	–	+
9	45	1:100,000	20	5	Large	+	+
10	44	1:100,000	20	5	Large	+	+
11	44	1:100,000	20	10	Large	+	+
12	43	1:100,000	20	10	Small	–	–

The copper bands of the canines in the right upper and lower jaws were filled with a mixture containing tetanus toxin and glucose, where the toxin titer in 8 cases (Nos. 1 to 4 and 9 to 12) was 1:100,000, and in 4 cases (Nos. 5 to 8) 1:10,000. In all cases the glucose concentration was 20 per cent. The bands on the opposite side were filled with a mixture containing tetanus toxin and fructose, where the toxin titer in 8 cases (Nos. 1 to 4 and 9 to 12) was 1:100,000, and in 4 cases (Nos. 5 to 8) 1:10,000. The fructose concentration in 8 cases (Nos. 1 to 8) was 20 per cent, in 2 cases (Nos. 9 and 10) 5 per cent, and in 2 other cases (Nos. 11 and 12) 10 per cent. The time of action varied between 17 and 45 hours.

RESULTS

Three of the experiments were totally negative, probably on account of too small an amount of pulp tissue (Nos. 3, 4, and 12).

Cases 1 and 2 were positive to both toxin-glucose and toxin-fructose, from which it may be concluded that fructose possesses the same capacity for increasing penetration as glucose. However, in Nos. 5 to 8 a detectable amount of toxin was present in the pulp of the teeth influenced by toxin-fructose despite the fact that the toxin titer was only 1:10,000. This shows that fructose has a greater capacity for increasing the toxin penetration than has glucose. (See Table I, Nos. 12 and 13.) Following this observation the fructose concentration was decreased and positive results were obtained with 5 per cent (Nos. 9 and 10) and 10 per cent (No. 11) fructose solutions.

We are not able to offer any explanation of these remarkable facts.

All experiments with toxin depend on factors in the animals and toxin stability. With the methods adopted a positive result may be obtained only on the presumption that an amount of toxin, sufficient for a biologic test after its passage through enamel and dentin, has been deposited in the pulp. Should the dogs be more than 12 months of age the amount of pulp tissue obtained might be too small and the quantity of toxin in the pulp too insignificant to be determined. Moreover, in older dogs increasing mineralization will render penetration difficult or even prevent it altogether. From personal experience we know that the ideal age lies between 7 and 12 months. In order to perform the experiments as effectively as possible, x-ray films of the teeth ought to be taken beforehand to get a clear idea of the pulp volume. Unfortunately we did not have the opportunity of doing so in all cases, and some experiments were therefore spoiled. Further, the stability of the toxin will play an important part. In some cases the toxin preparation used showed a very rapid decrease of its titer, thus threatening to damage the experiments.

Another condition on which a good result will depend is that the toxin must be in contact with the enamel for a sufficiently long time, probably at least 24 hours. This means that the animals must be kept under morphine for such a period. If they are highly morphine resistant it may cause some trouble as they must be injected each hour or two. In other cases the animals may be exceptionally morphine sensitive and die during the experiments.

3. The Influence of Different Sugar Solutions Upon Dye Penetration

Experiments with dyes in combination with different sugar solutions were carried out on 10 dogs.

The four canines were fitted with copper bands as described before. The bands on the right upper and lower teeth were filled up with different dyes in saline. On the opposite side they were filled with dyes in sugar solutions. (Table IV.)

The duration of exposure was in one case (No. 5) 3 hours. In the remaining cases it varied between 14 and 30 hours.

Table IV

Penetration of Dyes in Combination With Different Sugar Solutions

No.	Dye	Sugar solution	Sugar conc. (%)	Time of action (hr.)
1	Methylene blue	Glucose	30	3
2	Methylene blue	Glucose	30	27
3	Methylene blue	Glucose	30	14
4	Methylene blue	Glucose	25	30
5	Methylene blue	Glucose	20	24
6	Methylene blue	Fructose	25	17
7	Methylene blue	Saccharose	25	15
8	Toluidine blue	Glucose	20	26
9	Fuchsin	Glucose	25	29
10	Fuchsin	Glucose	25	30

After the animals were sacrificed, the teeth were removed and divided longitudinally in two halves. The section surfaces were ground and well polished. Microscopic observations were then made with reflecting light using Leitz Ultropak. For the photomicrography Anseo tungsten film was used.

RESULTS

No difference in the dye penetration could be established whether the teeth had been influenced by dyes in combination with sugar solutions or with saline. All dyestuffs had stained the enamel in the same parts of the teeth and to a similar extent. The penetration had occurred around the crown in its gingival third in a section approximately 2 mm. broad and along the lingual torus. (Fig. 1.)

Toluidine blue and fuchsin gave very diffuse stainings while, on the other hand, methylene blue staining was very intense.

Figs. 2 and 3 show the staining in higher magnification (×22). The strange figures seen are characteristic—Fig. 2 for the penetration in the gingival third and Fig. 3 for the staining along the lingual torus.

Of course the age of the dogs will have an influence on the staining intensity, and in teeth from older dogs the strange figures mentioned are either very ill-defined or are not seen at all.

Sugar solutions did not influence dye penetration. The picture was the same in teeth not influenced by sugar solutions. The same parts of the enamel

were stained; the degrees of penetration and the intensities of staining were similar. However, it should not be concluded that sugar solutions did not intensify a dye penetration in the same way as toxin. It might be assumed in these cases that the dyestuff in association with the organic matter of the enamel blocks the way for further passage of dye particles. For this reason, perhaps, the increased penetration that we had expected was not observed.

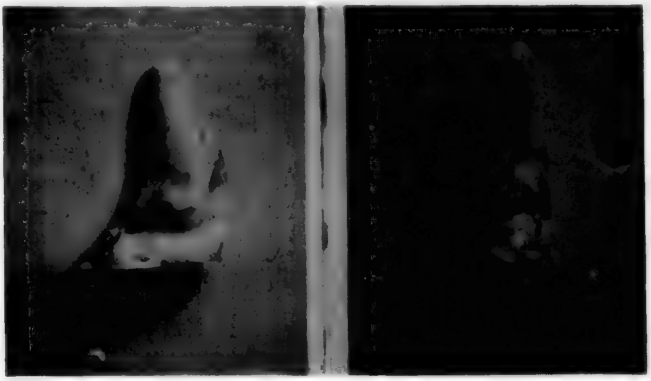

Fig. 1.

Fig. 2.

Fig. 3.

SUMMARY

An investigation was performed to study the influence of glucose, fructose, and saccharose upon the permeability of enamel to tetanus toxin and different dyes.

It was established that:

1. Addition of glucose to a mixture of tetanus toxin and broth will increase the toxin penetration, and there is a definite relation between glucose concentration and toxin penetration.

2. Fructose will increase the penetration of tetanus toxin to a higher degree than glucose.

3. Addition of glucose, fructose, and saccharose to methylene blue, toluidine blue, or fuchsin apparently has no enhancing effect upon the dye penetration. The experimental results, however, must not be considered as fully conclusive.

THE INFLUENCE OF TOPICALLY APPLIED FLUORINE ON THE PERMEABILITY OF ENAMEL

HELGE BERGGREN, ODONT.D., AND HARRY HEDSTRÖM, VET.M.D.

Department of Operative Dentistry, State Dental College and State Veterinary Medical Institute, Stockholm, Sweden

FEW odontologic problems have attracted as much interest as the fluorine problem, and few methods designed for checking caries have been met with greater optimism and expectation than topical fluorine application.

According to the abundant literature on this subject the most prevalent opinion on the action of fluorine seems to be that there is a chemical combination between the hydroxyapatite of the enamel and the fluoride on applying sodium fluoride to a well-polished enamel surface, resulting in an increase in the acid resistance of the enamel. It also has been stated that the newly formed calcium fluoride layer consists of smaller and more closely packed crystals than untreated enamel, giving the layer a decreased permeability. As far as was known to the authors this fact had not been established experimentally in vivo so that a practical study of the subject promised to be of considerable value.

MATERIAL AND METHODS

The experiments were carried out on 16 dogs.

The left upper and lower canines were topically treated with sodium fluoride in a way similar to that described by Knutson. After the teeth had been polished carefully with pumice powder and washed with alcohol, 2 or 4 per cent sodium fluoride solutions were applied over 30 to 90 minutes. (Table I.) The untreated canines on the opposite side served as controls.

After a lapse of a week all four canines were fitted with copper bands as described by Berggren[1] and Berggren and Hedström.[2] The bands were filled with a mixture containing tetanus toxin and fructose, where the toxin titer was 1:100,000 and the fructose concentration 20 per cent. The animals were kept under morphine for 38 to 44 hours and then sacrificed. As in previous investigations[1, 2] the penetration of toxin through the dental hard tissues was established by its presence in the pulp.

RESULTS

Toxin penetration occurred in both fluorine-treated and untreated teeth in 13 cases (Table I). The remaining three gave negative results in both groups of teeth. The roots of No. 10 were not fully developed, and too little pulp tissue was obtained to yield sufficient material for inoculation of mice. No explanation is offered of the negative results of Nos. 15 and 16.

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TABLE I

PERMEABILITY OF ENAMEL TO TOXIN AFTER TOPICAL FLUORINE APPLICATION

NO.	FLUORINE CONC. (%)	NUMBER OF PERIODS OF FLUORINE APPLICATION	MINUTES PER PERIOD	TOTAL PERIOD OF FLOURINE-APPLICATION (MIN.)	TIME OF ACTION OF TOXIN (HR.)	TOXIN TITER	FRUCTOSE CONC. (%)	PULP VOLUME	RESULTS	
									FLUORIZED TEETH	NON-FLUORIZED TEETH
1	2	6	5	30	44	1:100,000	20	Large	+	+
2	2	6	5	30	43	1:100,000	20	Large	+	+
3	2	6	5	30	42	1:100,000	20	Large	+	+
4	2	6	5	30	41	1:100,000	20	Large	+	+
5	4	1	30	30	41	1:100,000	20	Large	+	+
6	4	1	30	30	43	1:100,000	20	Large	+	+
7	4	3	15	45	43	1:100,000	20	Large	+	+
8	4	3	15	45	41	1:100,000	20	Large	+	+
9	4	3	15	45	38	1:100,000	20	Large	+	+
10	4	3	15	45	41	1:100,000	20	Small	−	−
11	4	2	30	60	42	1:100,000	20	Large	+	+
12	4	2	30	60	39	1:100,000	20	Large	+	+
13	4	2	30	60	41	1:100,000	20	Large	+	+
14	4	2	30	60	41	1:100,000	20	Large	+	+
15	4	3	30	90	42	1:100,000	20	Large	−	−
16	4	3	30	90	41	1:100,000	20	Large	−	−

DISCUSSION

Of the 13 cases in which positive results were obtained from treated as well as untreated teeth, the permeability of the enamel was not decreased despite the fact that the total period of fluorine application was never less than 30 minutes and the fluorine concentration in most cases was as high as 4 per cent. Even if a fluorine effect had been expected, so complete a permeability decrease that the mice inoculated with pulp suspension from the fluorine-treated teeth would survive while the others died was not anticipated. There was a possibility of observing a graduated course, from those instances in which the mice were poisoned to the point that they died as early as the day following the inoculation, through those cases in which the death did not occur for a few days, to those cases in which the animals had typical symptoms of tetanus but survived. It was established, however, that the degree of poisoning was the same in each group of nonimmunized mice inoculated with pulp suspension from the same dog, whether the pulp suspension was obtained from the fluorine-treated or the untreated teeth.

SUMMARY

Investigations have been performed to test the effect of topical fluorine application upon the permeability of enamel of dogs 7 to 12 months old. After application of a 2 or 4 per cent sodium fluoride solution to the canines, these teeth were exposed for 38 to 44 hours to a toxin-fructose mixture, where the toxin titer was 1:100,000 and the fructose concentration 20 per cent. The permeability of the dental enamel was unchanged by treating the teeth with fluorine.

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FLUORINE IN HUMAN TEETH STUDIED IN RELATION TO FLUORINE IN THE DRINKING WATER

F. J. McCLURE AND R. C. LIKINS

National Institute of Dental Research, Bethesda, Md.

IN A previous publication the senior author reported that there were no differences in the fluorine content of enamel and dentin of sound versus carious teeth and that fluorine differences between individual teeth would not explain the presence or absence of caries.[1] At the same time it was suggested that there was reason to believe "that an unusual dietary (particularly water-fluoride) exposure during the formative tooth life may increase the fluorine content of the entire dentition" and that such an increase in fluorine content might account for an over-all reduction in the dental caries experience. A considerable quantity of data has now been accumulated which gives some insight into the relation of fluorine in drinking water to the fluorine in dentin and enamel of sound and carious teeth. These data are the first to be presented in an attempt to answer the question as to the effect of drinking water containing an optimal quantity of fluorine (i.e., 1.0 to 1.2 ppm fluorine) on the fluorine in dentin and enamel of individuals continuously exposed to such a fluoride water.

In a previous publication[1] the data of other investigators relative to fluorine in human teeth were reviewed. Since that publication (June, 1948) two other contributions are known to have appeared in the literature.[2, 3] Harrison[2] found the mean fluorine content of New Zealand-born natives' teeth was 0.0042 per cent for the enamel of 89 subjects and 0.0060 per cent for the dentin of 8 subjects. "There was no significant difference between the fluorine content of the sound enamel of carious and non-carious teeth, all being low in this element."[2] New Zealand drinking waters contain 0.2 ppm or less fluorine (North Island)[4] and less than 0.05 ppm fluorine (South Island).[5] Fluorine in the drinking water of Switzerland as reported by Schmid[3] rarely exceeds 0.50 ppm and most potable Swiss waters contain slightly less than 0.10 ppm fluorine. Accordingly, fluorine in tooth-ash was found by Schmid[3] to be very low, i.e., rarely more than 0.010 per cent and most of the results show 0.003 to 0.009 per cent fluorine.

RESULTS

The procedures followed in the preparation and fluorine analysis of dentin and enamel are given in a previous publication.[1] The results of our most recent analyses together with some results which have appeared previously[1] are presented in Table I.

The teeth obtained from individuals living in McDuffie County, Ga., Washington, D. C., and vicinity, and Oslo, Norway, so far as was possible to ascertain, had been exposed to minimal quantities of water-borne fluorine. Among these three groups the average enamel-fluorine was 0.0092 per cent, 0.0100 per cent and 0.0079 per cent and dentin-fluorine was 0.0201 per cent, 0.0233 per cent,

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and 0.0086 per cent respectively. Individual results may vary considerably as shown by the maximum and minimum values in Table I. Excepting the low dentin figure for teeth from Oslo, these average values are regarded as fairly indicative of the amount of fluorine which may be present in dentin and enamel of individuals normally exposed to drinking waters whose fluorine content is of the order of 0.0 ppm to 0.3 ppm.

As noted in the footnote to Table I, the teeth from the north central Illinois area were obtained from individuals of unestablished residence history as well as of uncertain water-fluoride exposures. Although collected in a geographical area which has a history of mottled enamel,[6] there is little or no indication in these data of any elevation of fluorine in the dentin and enamel.

The data relative to teeth from Aurora, Ill., are particularly important since they relate to the effect of a fluoride drinking water which is caries preventive but will not produce any appreciable incidence of mottled enamel.

The Aurora data are broken down in Table I, according to the age of the individual, but these figures give no evidence of differences among the age groups. The data indicate, however, that there is a general elevation of fluorine in both dentin and enamel when the drinking water contains 1.1 to 1.2 ppm fluorine. The residence histories of these individuals were quite carefully checked and all the individuals are regarded as having had a continuous exposure to this fluoride water. Among these Aurora teeth there were 23 noncarious teeth which averaged 0.0403 ± 0.0008 per cent fluorine in the dentin and 0.0114 ± 0.0001 per cent fluorine in the enamel, and 78 carious teeth which averaged 0.0367 ± 0.0003 per cent fluorine in the dentin and 0.0147 ± 0.0001 per cent fluorine in the enamel. The number of analyses for sound teeth is limited and a comparison of sound versus carious teeth is of uncertain value.* As these Aurora, Ill., data now stand there is no suggestion that the carious teeth contained less fluorine than the noncarious teeth.

The data respecting the effect of Galesburg, Ill., water (1.9 ppm fluorine) are scanty indeed, owing to difficulties in obtaining teeth. Only a very few enamel analyses were obtained and are not reported. Most of these teeth were carious and it was often impossible to obtain sufficient enamel for analysis. The dentin-fluorine increased (average, 0.0425 per cent) but not as much as might be anticipated from 1.9 ppm fluorine in the drinking water.

The potable waters of the West Texas Panhandle area are consistently high in fluorine (2.5 to 5.0 ppm F). Exposure to these waters is very decidedly reflected in the fluorine results for dentin (0.0762 per cent) and enamel (0.0345 per cent) as shown in Table I.

Campagnano di Roma, Italy, is a well-known mottled enamel area and the twelve teeth from this area showed mild to moderate mottling. The dentin- and enamel-fluorine, however, are much higher in these teeth than would be anticipated from the mottled enamel diagnosis and from the fluorine in the drinking water. It seems likely, therefore, that during some earlier period in the lives

*The necessity of pooling teeth for a suitable analytic sample reduced the number of actual analyses to 13 in the case of sound teeth and 36 in the carious teeth. The morphologic types of teeth were similarly represented in both groups of teeth.

TABLE I

FLUORINE CONTENT OF TEETH FROM VARIOUS GEOGRAPHICAL AREAS AND FLUORIDE-WATER EXPOSURES

NUMBER OF INDIVIDUALS	GEOGRAPHICAL AREA AND TOOTH DESCRIPTION	FLUORINE IN DRINKING WATER* (PPM)	FLUORINE CONTENT OF DENTIN MAX. (%)	DENTIN MIN. (%)	DENTIN AVE. (%)	ENAMEL MAX. (%)	ENAMEL MIN. (%)	ENAMEL AVE. (%)
9	McDuffie County, Ga.	0.1						
	16 noncarious teeth		0.0480	0.0099	0.0216	0.0220	0.0051	0.0086
	13 carious teeth		0.0544	0.0143	0.0186	0.0193	0.0040	0.0097
33	Wash., D. C., and vicinity	0.0 – 0.2						
	224 noncarious teeth		0.0482	0.0113	0.0240	0.0203	0.0046	0.0102
	202 carious teeth		0.0517	0.0086	0.0227	0.0220	0.0042	0.0098
9	Oslo, Norway	0.2 – 0.4						
	27 noncarious teeth		0.0126	0.0050	0.0086	0.0178	0.0025	0.0079
44	North central Ill.	--						
	22 noncarious teeth		0.0368	0.0107	0.0300	0.0132	0.0045	0.0103
	33 carious teeth		0.0521	0.0069	0.0210	0.0173	0.0037	0.0093
31	Aurora, Ill. Age 8-25	1.1 – 1.2						
	36 carious and noncarious teeth		0.0702	0.0125	0.0360	0.0253	0.0064	0.0133
12	Age 25-45							
	35 carious and noncarious teeth		0.0632	0.0316	0.0361	0.0221	0.0048	0.0142
17	Age over 45							
	30 carious and noncarious teeth		0.0706	0.0253	0.0440	0.0200	0.0096	0.0140
13	Galesburg, Ill.	1.9						
	29 carious and noncarious teeth		0.0692	0.0261	0.0425	--	--	--
—	West Texas Panhandle Area	2.5 – 5.0						
	21 carious and noncarious teeth		0.1033	0.0550	0.0762	0.0564	0.0149	0.0345
12	Campagnano di Roma, Italy	(3.5)						
	12 carious and noncarious teeth		0.2139	0.1123	0.1589	0.0772	0.0386	0.0599
--	Chetopa, Kan.	7.6						
	7 noncarious teeth		0.1814	0.0809	0.1277	0.1059	0.0384	0.0658

*The sources of the data on the fluorine content of these drinking waters are as follows:

(a) McDuffie County, Ga. Four samples from different areas in the county were obtained in January, 1945, and were analyzed by Dr. Elias Elvove, National Institutes of Health.

(b) Oslo, Norway. Ten samples of water were supplied by Dr. Guterom Toverud in June, 1948, and were analyzed by the senior author.

(c) Washington, D. C. Various samples of water were analyzed by Dr. Elias Elvove and the senior author during the years 1935 to 1948.

(d) North central Illinois. The waters from this area are quite variable—see Deatherage, C. F., Klassen, C. W., and Weart, J. G.: Fluoride and Mottled Enamel in Illinois, *Illinois D. J.* **8**: 194, 1939. The uncertain and no doubt variable residence histories of these individuals does not permit any good estimate of their water fluoride exposures. The fluorine data on the teeth suggest, however, that the water-fluorine was probably not above 0.2 to 0.3 ppm.

(e) Aurora, Ill. See Reference 7.

(f) Galesburg, Ill. See Dean, H. T., Jay, Philip, Arnold, F. A., Jr.; McClure, F. J., and Elvove, Elias: Domestic Water and Dental Caries Including Certain Epidemiological Aspects of Oral L. Acidophilus, *Pub. Health Rep.* **54**: 862, 1939.

(g) West Texas Panhandle area. McClure, F. J.: Fluoride Domestic Waters and Systemic Effects, *Pub. Health Rep.* **59**: 1543, 1944.

(h) Campagnano di Roma, Italy. One water sample was shipped from Italy in February, 1946, by Dr. Isaac Schour of the University of Illinois School of Dentistry and analyzed by Dr. Elias Elvove. This result (3.5 ppm) is lower than would be anticipated from the results of the teeth analyses. Visintin and Gandolfo, however, [Il fluro in natura come agente morbigeno (Nota 1. Le acque ed il suolo di Campagnano di Roma. Estratto Dai Rendiconti, Dell Istituto Superiore di Sanita. Roma, **22**: 265, 1947] report only 0.61 to 1.17 ppm fluorine in six samples of drinking water from this area.

(i) Chetopa, Kan. See Reference 6.

of these Italian natives their drinking water contained appreciably more than 3.5 ppm of fluorine. Schour and Massler[6] also suggest that "a lowered nutritional status resulted in an increased prevalence and a higher degree of mottling in the teeth of children using a fluorine-containing communal water supply." Judging from our high fluorine results for these teeth from Italy, this might be true if water consumption was unusually high because of absence of milk from the children's diet.

The Chetopa, Kan., teeth were developed at a time when the water contained 7.6 ppm of fluorine and the mottled enamel index was 3.2 and 100 per cent of the population was affected.[7] All of the seven noncarious teeth showed moderate to severe mottling.

Sufficient data have not been accumulated for a full-scale survey of the effect of a wide range in water-borne fluorine on fluorine in teeth. A major difficulty in a study such as this is the necessity of ascertaining the individual's residence history before accepting teeth for analysis. The continuity of exposure (as well as the quantity of fluorine in the drinking water) is obviously a most important factor affecting the fluorine in teeth.

DISCUSSION

A number of reports appearing prior to this publication have shown that fluorine accumulates in dentin and enamel of mottled teeth, i.e., when there has been an exposure to a fluoride water for some considerable duration in the formative period of the teeth. These reports were reviewed in a previous publication.[1] Little or no information was presented in these previous studies, however, in regard to the concentration of fluorine in the drinking water to which these teeth were exposed. In these previous data, as well as in our own data, the correlation between severity of mottling and the fluorine content was not significant. These data have established the fact, however, that fluorine does accumulate in abnormal quantities in mottled teeth.

The quantity of fluorine in dentin and enamel of teeth which are nonmottled but which have been exposed continuously to a drinking water containing 1.1 to 1.2 ppm fluorine had not been studied prior to this report. It will be recalled that the dental caries experience among such teeth is about 65 per cent below normal and mottled enamel is practically absent.[8] Any attempt to explain this effect of fluorine on dental caries must take into account the fluorine in the enamel and dentin. In connection with the low incidence of dental caries in persons from Aurora, Ill., it was important, therefore, to ascertain the amount of fluorine in Aurora teeth as compared with the fluorine in "control" or "minimal-fluoride-exposure-teeth." It now seems apparent that 1.1 to 1.2 ppm fluorine in drinking water does cause an appreciable increase of fluorine in the dentin and enamel of teeth continuously exposed to such drinking water. In enamel this increase amounts to about 30 per cent and in the dentin to about 60 per cent. These increases are based on an average of 0.0102 ± 0.0004 per cent* fluorine in

*$P.E_M = \frac{0.6745\sigma}{\sqrt{N}}$

enamel of "control" or "nonfluoride teeth"[1] versus 0.0133 ± 0.0001 per cent fluorine in enamel of Aurora teeth and 0.0241 ± 0.0010 per cent fluorine in the dentin of "control" or "nonfluoride teeth"[1] versus 0.0385 ± 0.0002 per cent fluorine in the dentin of Aurora, Ill., teeth. The difference in enamel fluorine is 0.0031 ± 0.0004 per cent. The difference in dentin fluorine is 0.0144 ± 0.0010 per cent. Both differences are statistically significant.*

The importance of these data, in connection with theoretical considerations of caries etiology, lies in the fact that a reduction in dental caries due to consumption of a near optimal concentration of fluorine in drinking water is directly associated with an increase of fluorine in dentin and enamel. Thus the role of fluorine, as deposited during formative tooth life, remains a prominent factor to be considered in solving the question of resistance to dental caries.

CONCLUSIONS

The teeth of individuals continuously exposed to drinking water containing 0.0 to 0.3 ppm fluorine may be expected to contain approximately 0.0100 per cent fluorine in the enamel and 0.0240 per cent fluorine in the dentin. A near dental-optimal quantity of fluorine in Aurora, Ill., drinking water (i.e., 1.1 to 1.2 ppm) resulted in 0.0133 per cent fluorine in the enamel and 0.0385 per cent fluorine in the dentin. These additional quantities of fluorine in dentin and enamel are associated with a marked reduction in the dental caries experience, but dental fluorosis is not an endemic health problem among such teeth. Data are presented to show the effect of other higher levels of fluorine in drinking water on the fluorine in human dentin and enamel.

The authors are indebted particularly to the dentists of Aurora, Ill., who cooperated in collecting Aurora teeth for analysis; to dentists in Galesburg, Ill., and the Texas Panhandle area; and to Dr. I. Schour of the University of Illinois School of Dentistry, for the teeth from Italy.

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*A significant statistical difference is one which is in excess of

$$3\sqrt{PE_{Mx^2} + PE_{My^2}}$$

THE SOLUBILITY OF NORMAL AND FLUORIDIZED ENAMEL

PHILIP H. SUESS AND L. S. FOSDICK

Chemistry Department, Northwestern University Dental School, Chicago, Ill.

SODIUM fluoride, when topically applied to the teeth, will cause a marked reduction in the caries activity.[1] This treatment is now used extensively by public health services and by general practitioners. On the basis of the chemical properties of the fluoride ion and upon the current concept of dental caries, the effect may be due to an enzyme inhibition or to a change in the solubility of the inorganic portions of the tooth. It is known that the fluoride ion will inhibit the hydrolysis of phosphoric esters and hence interfere with the formation of acids from glucose.[2, 3] This inhibition of the hydrolysis of esters, however, requires relatively high concentrations of the fluoride ion before a complete cessation of acid formation is observed. A slight retardation of acid formation is observable in concentrations of 1 ppm.[4] In order to cause a retardation of acid formation, the fluoride ion must be in solution. This precludes a permanent or lasting effect by the topical application of fluorides. Insofar as the topical application of fluorides to the surface does produce a lasting effect, it is quite probable that the enzyme inhibitory action is of minor importance. In view of this, it is quite probable that the observed effect is due to a change in acid solubility of the enamel. Many investigators have found that fluoroapatite is less soluble than hydroxyapatite in acid solution.[5, 6, 7] Fluoridized human enamel acts very similarly to fluoroapatite. The solubility of fluoroapatite and fluoridized tooth enamel has been studied quite extensively in various acid solutions,[8] but insofar as the type of acid used is of less importance than the reaction of the solution, it was thought desirable to determine solubility in relation to the pH of the solvent and to formulate the conditions under which the fluoride ion would protect the teeth.

EXPERIMENTAL

Molar teeth were selected at random, dried in vacuuo, and the enamel caps removed. The enamel chips, mostly from the proximal surfaces, were examined with a hand lens and any residual dentin was removed by means of a diamond stone. The clean enamel was ground with a mortar and pestle and passed through a 200 mesh sieve. Only that portion that passed the 200 mesh sieve and failed to pass a 325 mesh sieve was used. This yielded a relatively homogeneous product so that aliquot portions would have a comparable surface exposed. One-half of the 22 grams of enamel were allowed to

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stand in 2 per cent sodium fluoride solution at 37.5° C. for three hours, at which time the mixture was filtered and washed with 200 c.c. of distilled water in 50 c.c. portions. The fluoridized enamel was then air dried, after which both the normal and fluoridized enamel were stored in a vacuum desiccator to constant weight. A fluorine analysis* showed that the normal enamel contained 126.5 ppm. F. while the fluoridized enamel contained 1,434.6 ppm F. The calcium phosphorus ratios were 2.01 and 2.05 respectively. The relatively constant calcium-phosphorus ratio precluded the possibility of the fluoride appearing as calcium fluoride, so in all probability most or all of the fluoride was in the form of fluoroapatite.

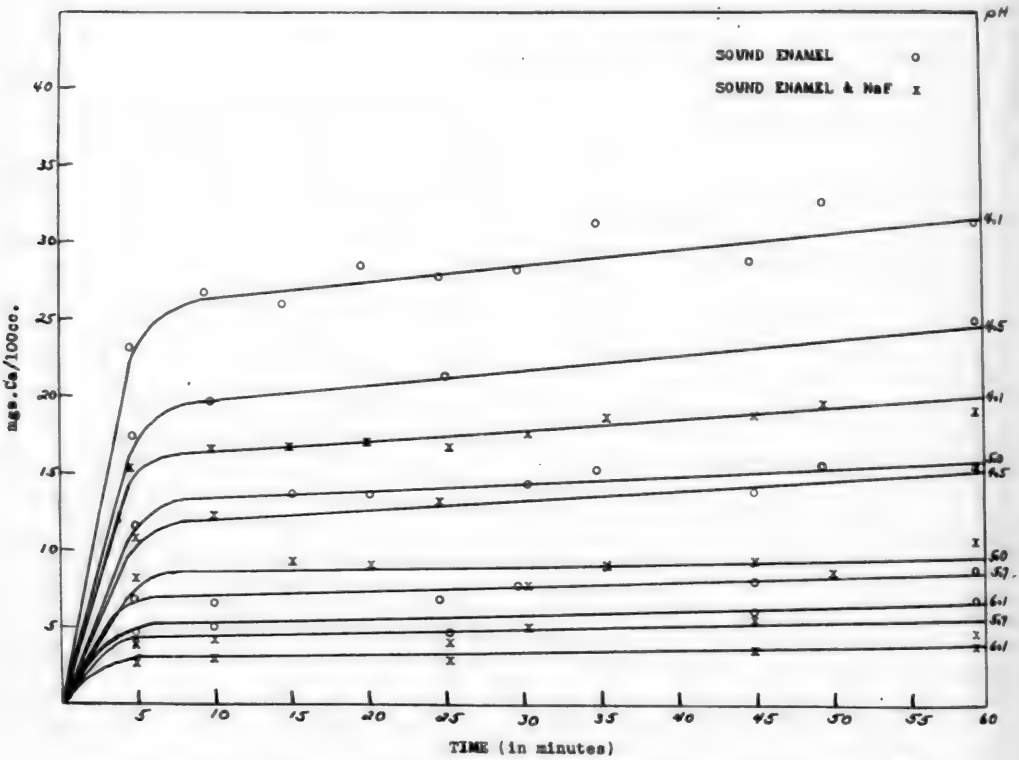

Fig. 1.—The rate of solubility of tooth enamel in buffer solutions as determined by the calcium concentration of the solvent.

Buffers were prepared at 0.5 pH value intervals from pH 4 to pH 7. The first five were phthalate buffers and those for pH 6.5 and pH 7 were maleate buffers. The buffers were checked by means of a glass electrode. In order to obtain the rate of solution of the two types of enamel, two series of experiments were performed. In the first series the rate of solution in each

*The authors wish to thank D. J. Martin of the Department of Health, Evanston, Ill., for the fluorine analysis.

of the buffers was obtained; in the second series the time was held constant and the amount of dissolved calcium and phosphorus was compared with the pH of the solvent. For the first series 100 mg. of the powdered enamel were added to 20 c.c. of the buffered solution. The container was capped and shaken for sixty minutes. Samples were removed at intervals and filtered for calcium analysis, and since only four samples could be obtained from each vial, enough containers were used so that an analysis at each five-minute interval was secured. In each case the analysis was done in duplicate.

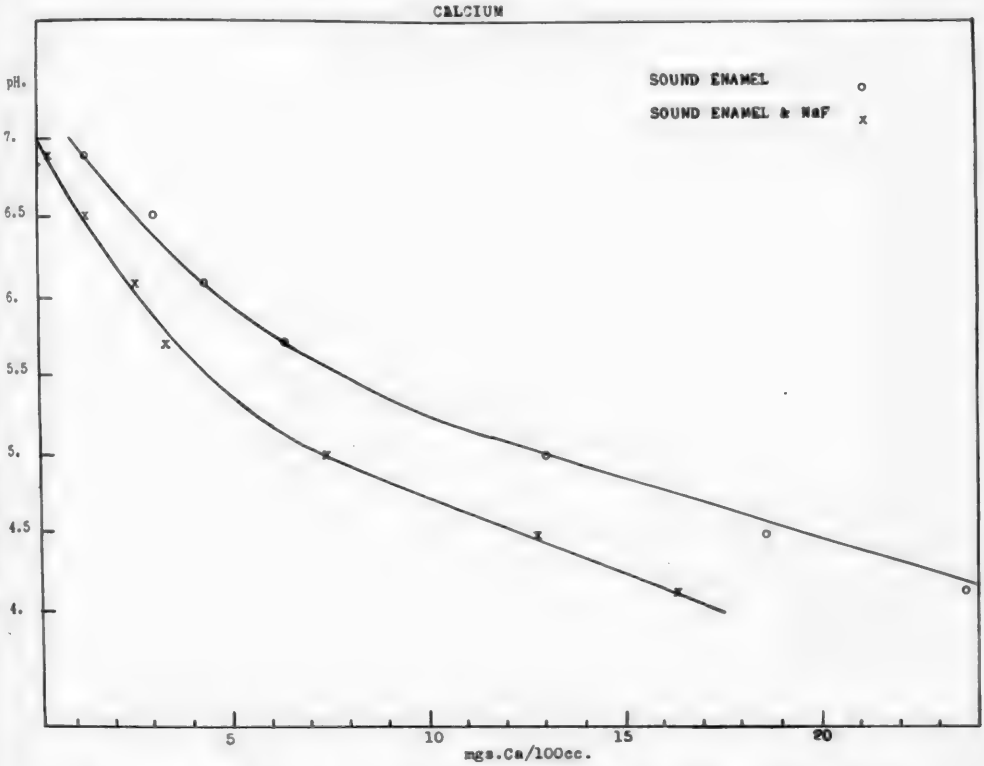

Fig. 2.—The effect of pH on the calcium concentration of the solvent (15-minute contact).

It was found that the equilibrium was approached after a fifteen-minute time interval. In the second series of experiments this period of contact of the solvent with the enamel was allowed. In this series 50 mg. of enamel were shaken with 10 c.c. of the buffer solution for fifteen minutes, after which the sample was filtered and the solvent was analyzed for both calcium and phosphorus. In all cases the calcium was determined by the Clark-Collip modification of the Kramer-Tisdall method.[9] The phosphorus was analyzed according to the Briggs modification of the Fisk-Subbarow method.[10] A Coleman spectophotometer was used for the determination of the transmission.

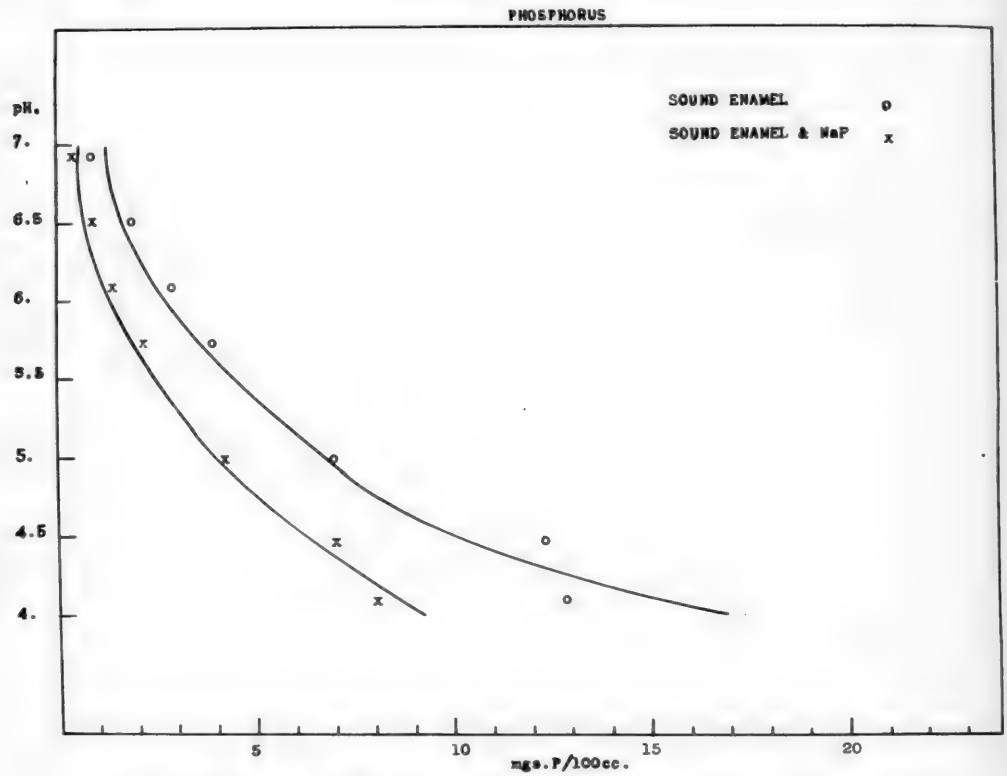

Fig. 3.—The effect of pH on the phosphorus content of the solvent (15-minute contact).

DISCUSSION

The results are shown in Figs. 1, 2, and 3. As can be seen by the various curves, it would appear that the fluoridized enamel solubility curves are similar in slope to the normal curves with the exception that they are about 0.5 pH units lower. Thus, if we assume that the normal enamel will start to decalcify at pH 5.2, then the fluoridized enamel will start at 4.7; or, if the normal enamel will start to dissolve at pH 5.5, then the fluoridized teeth are protected to a pH of 5.0. When one considers the acidity of the tooth surface after the ingestion of fermentable carbohydrates, it becomes obvious that in a large number of cases the fluoridized teeth are amply protected against decalcification. The pH of the tooth surface is ordinarily about the same as that of saliva. At this reaction the teeth will not declacify as they are amply protected by the calcium and phosphorus concentrations normally found in the saliva. When concentrated sugars are added to the tooth surface, almost invariably acids are formed. The organic covering of the tooth ordinarily protects the teeth against the action of acids in that this organic covering is an efficient buffer. If, however, the buffering capacity of the plaque is low or if the amount of acids that is formed is large, then the tooth surface becomes

more acid. Under ordinary conditions the anterior teeth may have a pH as low as 5.0 on the surface of the enamel, but only on rare occasions does the acidity increase to points where the teeth rapidly decalcify. Under normal circumstances the pH of the anterior tooth surface rarely or never approaches 4.7 so that the fluoridized anterior tooth should be almost completely protected against caries. The surface of the posterior teeth, however, is better protected from the saliva so that values as low as pH 4 are not rare and pH values below 5 are quite frequent. Under ordinary circumstances many of the posterior teeth become sufficiently acid for a decalcification to take place, but if fluoridized, this acid potential does not ordinarily occur. Under these circumstances the posterior teeth would be partially protected. However, in cases of rampant caries wherein the hydrogen-ion concentration on the tooth surface will usually reach a pH of 4 after the application of sugar, the fluoride ion would offer very little protection. Rather than prevent decay, it would simply delay the formation of a cavity. Thus, in cases of rampant caries, fluoridization of the teeth would simply provide a delaying action, while in cases of mild activity almost complete protection against caries would be present. If the foregoing results and interpretations are correct, one would expect that the topical application of fluoride would provide almost complete protection to the anterior teeth and would provide less protection to the posterior teeth. Furthermore, cases of mild caries activity would be provided complete protection, while rampant caries would have no or only a small amount of protection. Unfortunately, the clinical studies that have been performed to date do not provide data from this point of view. It would be of interest to examine the data in relation to the normal caries activity of the individual.

SUMMARY

The solubility of normal and fluoridized enamel in buffered solutions have been studied. An explanation concerning the effect of fluoridization has been offered.

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INVESTIGATIONS ON THE METABOLISM OF FLUORIDE

I. The Determination of Fluoride in Blood

FRANK A. SMITH AND DWIGHT E. GARDNER

With the Collaboration of M. J. Voss and the Technical Assistance of D. Wing, P. Dunn, J. Figueras, and J. W. Keating

Division of Pharmacology and Toxicology, Department of Radiation Biology, School of Medicine and Dentistry, The University of Rochester, Rochester, N. Y.

THIS paper reports the analytic method used in the recently published investigation of the blood fluoride concentration of persons drinking water containing 1 ppm fluoride[1] Studies of the biologic effects of fluorides have centered around a few important topics[2-7]; however a survey of blood fluoride concentrations reported for different methods[8] showed erratic results, some as high as 460 μg F per 100 ml. A brief investigation of various ashing procedures indicated that the principal source of the variability was to be found in this initial preparation of the sample. Using a variety of alkaline fluoride fixatives, 50 ml. samples of blood were made alkaline, evaporated to dryness, and ashed overnight either at 350° or 575° C. The fluoride was distilled from a perchloric acid solution of the ash and then determined in the distillate by a modification to be described later of the thorium-alizarin fluoride titration. Typical analytic data are shown in Table I; the recoveries are erratic and low. Because blood contains appreciable quantities of iron capable of forming volatile iron fluoride complexes or compounds, the importance of iron was established. Recovery studies were done as before, using an amount of iron as ferric chloride equivalent to the iron content of 50 ml. of blood, and sodium carbonate as fixative. Approximately 70 per cent of the added fluoride was lost, indicating that the low recoveries (Table I) could be attributed, at least in part, to volatile iron fluorides. These difficulties were obviated when the double distillation procedure of the Association of Official Agricultural Chemists[9] was used.

Distillation.—A general description of the reagents, apparatus, and procedure for the fluoride distillation as done in this laboratory has been given by Flagg.[10] For the analysis of blood, a 50 ml. sample of oxalated whole blood is introduced directly into a 200 ml. distilling flask and the distillation carried out in the usual manner, using concentrated sulfuric acid rather than perchloric acid for the first distillation. A total of 140 ml. of distillate is collected. The distillate is evaporated to dryness in platinum, using calcium oxide as a fixative, ashed 15 to 16 hours and redistilled as before, except that perchloric acid is used as an acidifying agent and 125 ml. of distillate are collected.

Titration.—The salt-acid thorium method of Williams[11] offers at least two improvements over the usual thorium-alizarin back titration[9]: (a) the use of a

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TABLE I

DIRECT ASHING PROCEDURES. RECOVERY OF FLUORIDE ADDED TO BLOOD

FIXATIVE	ASHING TEMPERATURE (° C.)	MICROGRAMS F ADDED	PER CENT F RECOVERED
CaO	575	25	78.0
		50	77.0
KOH	575	100	35.7
Na_2CO_3	350	20	58.0

thorium solution having a constant fluorine equivalence, and (b) the use of a single titration against a permanent color standard. Certain modifications were made of the original procedure as described by Williams for convenience in routine use. Three of these may be mentioned: (a) Reagent concentrations were adjusted to insure a constant thorium-fluorine ratio with the grade of reagent available; (b) neutralization of the sample was accomplished in a manner similar to that of the older "back-titration" procedure which is more convenient and rapid than that proposed by Williams[11]; and (c) the present titration method eliminates the cumbersome procedure of preparing a new color standard from the stock solution for each analysis; instead both titration and standard end point solution volumes were limited and a permanent color standard was used.

Recovery.—The mean recovery in 26 titrations of known quantities of fluoride ranging 5 to 80 μg was 99 per cent; the range of recovery in per cent was 94 to 105. The standard deviation in per cent was 2.3.[12]

Reagents.—All reagents are Baker's chemically pure grade unless otherwise specified. Reagents were prepared with doubly distilled water obtained from alkaline permanganate.

Acidified thorium nitrate. Dissolve 0.134 g of $Th(NO_3)_4 \cdot 4H_2O$ in water, add 7.5 ml. of 1.00N HCl and dilute to 1 liter. This solution is standardized against the sodium fluoride solution by titrating, as described below, known quantities of fluoride with thorium solution. One milliliter of the thorium solution is equivalent to approximately 5 μg of fluoride. Blank titrations on doubly distilled water require 0.98 to 1.02 ml. of thorium solution. The magnitude of the blank titration and the equivalence of the thorium to fluoride will vary with different grades of reagent and for different analysts.

Acid indicator. Dissolve 0.020 g of monosodium alizarin sulfonate (alizarin red S)* in water, add 16.1 ml. of 1.00N HCl and dilute to 200 ml.

Salt solution, 2N sodium chloride.

Hydroxylamine hydrochloride.† Dissolve 2 g of $NH_2OH \cdot HCl$ in water and dilute to 200 ml.

p-Nitrophenol indicator.† Dissolve 0.250 g of *p*-nitrophenol in 12.5 ml. of 95 per cent ethyl alcohol and add 37.5 ml. water.

Hydrochloric acid. Solutions of 1.00N and 0.05N are required.

Sodium hydroxide. A 1.0N solution is required.

*National Aniline and Chemical Company, New York, N. Y.
†Eastman Kodak Company, Rochester, N. Y.

Permanent color standard. Prepare a stock solution containing 3 ml. of 10 per cent hydrochloric acid, 42.19 ml. of 2 per cent cobaltous chloride, and 30 ml. of 0.1 per cent potassium chromate and dilute to 100 ml. To 3.2 ml. of this stock solution, add 67 ml. of distilled water in a tall-form Nessler tube. Keep the tube containing the permanent standard tightly stoppered when not in use.

Standard sodium fluoride. A stock solution is prepared by dissolving 0.221 g of spectroscopically pure NaF* in water and diluting to 1 liter. Dilute 100 ml. of the stock solution to 1 liter to obtain a solution containing 10 μg of fluoride per milliliter. This solution is used for standardization.

Apparatus.—Erlenmeyer flasks, 125 ml.

Tall-form (100 ml.) Nessler tubes which have been calibrated to contain 70 ml.

Daylight fluorescent lamp.

Titration Procedure.—In a 125 ml. Erlenmeyer flask are placed 0.05 ml. of 1.0N sodium hydroxide solution and 2 drops of *p*-nitrophenol indicator solution. A 50 ml. aliquot of the distillate is added to the flask and the solution neutralized against a white background with 0.05 N HCl to a faint tinge of yellow. From burettes are added (a) 1 ml. of $NH_2OH \cdot HCl$ to reduce any chlorine present from breakdown of the $HClO_4$, and (b) 10 ml. of 2N NaCl to control errors due to variations in the total ionic concentration. Two milliliters of the acid indicator solution are now added; this addition will adjust the final solution pH to 2.7 which is optimum for the titration. The solution is titrated with the acidified thorium nitrate solution against a daylight fluorescent illumination, using a 5 or 10 ml. microburette for delivery of the titrating solution. When the faint pink of the thorium lake appears, the solution is poured into a tall-form Nessler tube calibrated at 70 ml., and the flask is rinsed with 5 ml. of doubly distilled water. The rinsings are added to the solution in the Nessler tube, and the titration continued until the solution matches the color of the permanent standard when viewed under the same conditions in a similar tube. The matching of sample and standard is done against a daylight fluorescent lamp, by looking down through the depth of the solution. The final volume of the titrated solution should be 70 ml. $\pm$ 1 ml.

It is essential that the final volume of the solution being titrated and the volume of the permanent standard be nearly equal, in order that only one permanent standard of constant volume may be used for all samples titrated. This factor may be readily controlled by proper choice of the aliquot to be titrated. The aliquot may be of any volume up to and including 50 ml. and is chosen either on the basis of previous experience or by a preliminary pilot titration. Aliquots of less than 50 ml. are made up to this volume with doubly distilled water, allowing for the approximate volume of thorium nitrate which will be required.

The fluoride content of the sample is obtained from a recovery curve which converts the calculated amount of fluoride to the corrected micrograms F per sample.

*Aluminum Company of America, New Kensington, Pa.

Determination of Reagent and Procedure Blank Value.—Ideal conditions for the determination of the reagent and procedure blank value would require that the analysis be performed on blood samples known to be completely free from fluoride. The apparent fluoride content could then be attributed to the reagents, to intrinsic errors in the method, and to unknown factors present in the blood itself. Because it was impossible to obtain blood known to be free of fluoride, it was felt that a nearly correct approximation to the true blank value could be obtained by the separate analysis of a second portion of the sulfuric acid distillate of the blood (all of the fluoride should have been removed in the first portion of distillate). If serious contamination with fluoride from the reagents does occur, it was probably introduced subsequent to this point. Inasmuch as the recovery curve to be described includes suitable compensations for the reagent and procedure blanks, it obviously will be necessary to prepare a recovery curve for each lot of reagents. In practice, different lots of calcium oxide have proved to be the worst offender in causing variability in this respect; samples of this reagent from various sources have been found to contain from 3 to more than 80 μg of fluoride per gram. In practice, this difficulty can be obviated to a considerable degree by setting aside several pounds of calcium oxide, from the same lot number, for exclusive use in blood fluoride determinations. Approximately 350 to 400 analyses can be made per pound of oxide.

That all of the fluoride present in a 50 ml. sample of blood is removed in the first 140 ml. of distillate was shown by experiments in which 150 μg of fluoride were added to 50 ml. samples of blood. The sulfuric acid distillate was taken in several successive portions, each of which was then analyzed separately by evaporating to dryness in the presence of lime, ashing and distilling from perchloric acid as usual. It was found that approximately 98 per cent of the total fluoride recovered was found in the first two portions of distillate totaling 150 ml.; approximately 92 per cent of the total was found in the first 100 ml.

Accordingly, a series of analyses was done in which the sulfuric acid distillate from 50 ml. samples of beef, lamb, and dog blood was taken as two successive 140 ml. portions. Each portion of distillate was then analyzed separately by perchloric acid distillation after first evaporating and ashing. The fluoride contents of the first distillates are considered to be the normal fluoride values, plus the blank (Table II). The fluoride contents found for the second fractions are considered to be the blank for the reagents and procedure; the average value was 7.2 μg F $\pm$ 1.7. These values are used in the preparation of the recovery curve described below.

To determine whether there was any change in the blank value by unknown factors in the blood, distillations from 50 ml. portions of blood were compared with those from the same quantities of doubly distilled water. In these analyses several successive portions of distillate were taken during the sulfuric acid distillation; each portion was analyzed separately as before. The values for the second and third portions taken in the presence of blood were constant and not significantly different from those using distilled water or from the value of 7.2 μg F $\pm$ 1.7 found previously.

TABLE II

DETERMINATION OF THE REAGENT AND PROCEDURE BLANK AND BLANK PLUS BASE LEVEL IN THE PRESENCE OF BLOOD

FIFTY MILLILITER BLOOD SAMPLES; TWO PORTIONS OF SULFURIC ACID DISTILLATE TAKEN

SPECIES	FIRST PORTION DISTILLATE (BASE LEVEL PLUS BLANK)			SECOND PORTION DISTILLATE (REAGENT PLUS PROCEDURE BLANK)		
	NUMBER OF DET'NS	MEAN	ST. DEV.	NUMBER OF DET'NS	MEAN	ST. DEV.
Lamb	4	6.3	0.27	4	6.1	
Beef	4	8.1	1.94	4	9.7	
Beef	12	9.7	1.51	5	7.9	
Dog	4	10.5	1.55	3	6.1	
Dog	1	12.1	–	0	–	
Dog	6	8.8	0.87	6	6.3	
For all samples					7.2	1.7

Preparation of Blood Fluoride Recovery Curve.—A series of analyses was done in which known amounts of fluoride ranging from 5 to 70 μg were added to 50 ml. portions of animal blood (Table III). In next to the last column of Table III the micrograms of fluoride found in the first 140 ml. of distillate have been corrected by subtraction of the value for the combined base level and blank (Table II) for the appropriate species.

By the method of maximum likelihood,* the equation of the line best fitted to the data obtained was found to be:

$$x = 1.212\,y - 8.739$$

where y represents the micrograms of fluoride found and x represents the micrograms of fluoride actually present (or added).† The corrections made by

TABLE III

RECOVERY OF FLUORIDE ADDED TO BLOOD

SPECIES	NUMBER OF DET'NS	F ADDED (X)	MEAN F FOUND (Y)	MEAN BASE LEVEL PLUS BLANK FOR SPECIES AND SAMPLES (Z) (CF. TABLE II)	MEAN MICROGRAMS F FOUND CORR. FOR MEAN BASE LEVEL AND BLANK (Y)-(Z)	MEAN % F RECOVERED*
Lamb	4	5.0	11.4	6.3	5.1	
Beef	4		12.9	9.7	3.2	
Beef	3		15.6	12.5†	3.1	78.0
Lamb	3	25.0	28.8	6.3	22.5	
Dog	1		40.6	10.5	30.1	
Beef	3		31.2	8.1	23.1	
Beef	4		28.8	9.7	19.1	
Dog	1		32.8	12.1	20.7	88.0
Lamb	2	50.0	49.2	6.3	42.9	
Dog	1		55.9	10.5	45.4	
Beef	4		50.1	8.1	42.0	
Beef	4		48.8	9.7	39.1	
Beef	6		53.4	12.5†	40.9	82.4
Lamb	6	70.0	66.7	6.3	60.4	86.3

*Calculated from total number of determinations done at each level.
†Different beef blood from those listed in Table II.

*The statistical treatment of these data was performed by S. Lee Crump, Ph.D., Chief of Statistics Section, University of Rochester Atomic Energy Project.

†The equations representing the upper and lower 95 per cent confidence limits for a single determination on an unknown blood are $x = 1.212\,y + 0.100$ and $x = 1.212\,y - 17.500$, respectively.

the equation given above are not a constant correction of approximately 20 per cent, but the application of a constant correction plus a percentage correction, this latter component varying in magnitude with the amount of fluoride being determined. The mean percentage recovery of the fluoride added at each level is shown in Table III. As is to be expected, a lower recovery is found for the lower level of fluoride present. These recovery data indicate the necessity for using as large volumes of blood as are practicable. Application of the procedure is necessarily limited to investigations in which pooled blood samples are acceptable, or in which sufficiently large animals can be used to permit the taking of 50 ml. samples. If high fluoride levels are expected, and smaller volumes of blood are taken, a new recovery curve must be developed for the smaller volume.

RESULTS

To date 84 blood fluoride analyses have been obtained on 35 normal dogs and 20 analyses on 13 normal rabbits.* The dogs were fed once daily and were bled two hours before feeding; a minimal interval of two days intervened between any two successive bleedings on the same dog. The rabbits, which were fed ad libitum, were bled twice, by heart puncture, at two-week intervals. Blood fluoride levels are very low (Table IV). Thus, 81 per cent of the values for dog blood and 80 per cent of those for rabbit blood are below 10 μg F per 100 ml. Within the range of 10 to 20 μg F per 100 ml. are 12 per cent of the dog samples and all the remaining rabbit bloods. No reason can be offered to account for the extremely high values found for several of the dog analyses.†

TABLE IV

DISTRIBUTION OF VALUES FOR THE BLOOD FLUORIDE CONTENT OF NORMAL DOGS AND RABBITS

RANGE (μ G F PER 100 ML.)	DOGS		RABBITS	
	NUMBER	PER CENT	NUMBER	PER CENT
0.0- 2.4	26	31	6	30
2.5- 4.9	9	11	5	25
5.0- 9.9	33	39	5	25
10.0-14.9	9	11	4	20
15.0-19.9	1	1		
20.0-24.9	2	2		
25.0-29.9				
30.0-34.9	1	1		
35.0-39.9	1	1		
40.0-44.9				
45.0-49.9				
50.0-54.9	1	1		
55.0-59.9				
60.0-64.9				
65.0-69.9	1	1		

One specimen of lamb blood and two specimens of beef blood (Table II) contained 0.0, 2.2, and 6.0 μg F per 100 ml. respectively.

*Food analyses showed the diet of the dogs to contain 13 ppm of fluoride: that of the rabbits contained 3.4 ppm. Drinking water contained 0.06 ppm of fluoride.

†It was noted, however, that the four samples containing more than 30 μg F per 100 ml. were initial samples obtained on dogs shortly after they were brought into our colony. The fluoride contents of their previous diets are unknown.

SUMMARY

1. The adaptation of standard methods of fluoride analysis to the determination of fluoride in blood is described. A statistical evaluation is made of the precision of the over-all method.

2. Values are given for the fluoride content of blood from several species of animals. The values for normal dogs and rabbits are comparable and, in most cases, are less than 10 μg of fluoride per 100 ml. of blood.

We are pleased to acknowledge our indebtedness to H. C. Hodge and to H. E. Stokinger for helpful suggestions and criticisms.

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AN EVALUATION OF VARIOUS TECHNICS FOR REDUCING DECALCIFICATION OF ENAMEL

II. In Matched Enamel-Dentin Fragments

HUBERT B. PALMER, D.D.S., SUSIE OVERSTREET, B.S., AND MARILYN SACKS, B.S.

Department of Dental Research, Dental Division, Army Medical Department Research and Graduate School, Army Medical Center, Washington, D. C.

PALMER, Overstreet, and Sacks[1] have shown that when half the crown of an extracted molar tooth was treated with either an acidulated fluoride solution, a 2 per cent sodium fluoride solution, or a Zin-Ferro-Nitrate solution and immersed in broth seeded with *Lactobacillus acidophilus*, decalcification of enamel was reduced.

Since Palmer, Overstreet, and Sacks[1] have shown that teeth (treated and untreated) vary in their resistance to decalcification, a method for studying various agents for reducing decalcification which would utilize enamel-dentin fragments of the same tooth for both experimental and control purposes has been indicated. Such a method was used in this investigation, which greatly reduces variables in teeth noted in a previous study[1] and affords a more exacting technic for making a comparative evaluation of the solutions to be tested.

Cheyne,[2] Bibby,[3] Knutson and Armstrong,[4, 5, 6] Knutson, Armstrong, and Feldman,[7] and Jordan, Wood, Allison, and Irwin[8] have reported a decrease in the incidence of dental caries in groups of patients following the employment of a method utilizing certain fluoride solutions. Klinkenberg and Bibby[9] have reported a decrease in the incidence of dental caries for groups of individuals between 18 and 40 years of age whose teeth had been treated with solutions of 1 per cent sodium fluoride and 0.06 per cent lead fluoride. Crawford,[10] Younger,[11] and Gillard, Boelsche, and Summers[12] have reported a decrease in the incidence of dental caries in groups of patients following the employment of a method utilizing various pharmaceuticals in combination.

The purpose of this investigation has been twofold: (a) To confirm or refute findings of a previous report[1] that both an acidulated fluoride solution* and a 2 per cent sodium fluoride solution were equally effective for reducing decalcification† of enamel caused by growth products of *L. acidophilus* in vitro. (b) To confirm or refute findings[1] that the Zin-Ferro-Nitrate* solutions were less effective for reducing decalcification of enamel in vitro than were either an acidulated fluoride solution or a 2 per cent sodium fluoride solution.

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*A description of the solutions used, namely the acidulated fluoride solution and the Zin-Ferro-Nitrate solutions, will be found in a previous report.[1]

†Decalcification of dental tissue as referred to in this report is based upon the difference noted between the original and final weight of the enamel-dentin fragment following exposure to *L. acidophilus*.

METHOD OF STUDY

One hundred and two erupted, noncarious molar teeth extracted from 81 adults were thoroughly cleaned with scalers, rubber cups, and pumice. With the aid of separating disks the crown of each tooth, after being removed from the root at the cemento-enamel junction, was cut into four similar parts known as fragments A, B, C, and D. The enamel-dentin fragments were then washed for a few seconds in running tap water in order to remove any tooth dust or foreign particles present. The 408 fragments were then dried at 42° C. for six hours after which they were individually weighed and divided into twenty-five groups. Each group with the exception of one was represented by sixteen fragments from four teeth. Thus the groups consisted of four A fragments, four B fragments, four C fragments, and four D fragments. Group 195, the exception, represented six teeth and consisted of six A fragments, six B fragments, six C fragments, and six D fragments. In twelve groups numbered 166 to 177 inclusive the A fragments served as controls, none being treated. The cut enamel surfaces and exposed dentin surfaces of fragments B, C, and D of such groups, however, were first covered with paraffin, after which the fragments were treated with a test solution. The exposed (unwaxed) enamel surfaces of fragments B were covered with absorbent cotton saturated with the acidulated fluoride solution. The cotton was kept moist and allowed to remain upon each fragment for thirty-five minutes. The cotton was then removed and the tooth allowed to dry for five minutes. The exposed enamel surfaces of fragments C were similarly treated with chemically pure 2 per cent sodium fluoride solution. The exposed enamel surfaces of fragments D were treated with the Zin-Ferro-Nitrate solutions which were applied according to the method described in previous report.[1] Following the treatment of all the fragments with their respective test solution the paraffin covering on each fragment was removed.

In thirteen groups numbered 178 to 183 inclusive and 189 to 195 inclusive, fragments A, B, C, and D served as a dual control, none being treated. With the aid of stainless steel wire and perforated glass containers, each of the 408 fragments was placed in an individual test tube containing 10 c.c. of 70 per cent ethyl alcohol for twenty minutes (control studies following the treatment with alcohol showed no bacterial growth). All the fragments were then placed in individual test tubes and immersed under 10 c.c. of glucose-serum broth pH 7.0 seeded with 0.5 of 1 c.c. of 48-hour broth culture of *L. acidophilus* oral strain 12B. Incubation at 37° C. for thirty days followed. Fragments were transferred to new broth cultures on Monday, Wednesday, and Friday of each week. Following the thirty-day incubation period all the fragments were placed in alcohol for twenty minutes, washed, dried at 42° C. for six hours, and weighed.

RESULTS

Fragments B treated with an acidulated fluoride solution and fragments C treated with a 2 per cent sodium fluoride solution consistently showed in ten of the twelve groups a lower percentage mean weight loss than did the un-

treated fragments A or the fragments D treated with the Zin-Ferro-Nitrate solutions (Table I). For example, in Group 166 fragments treated with an acidulated fluoride solution showed a mean weight loss of 21.08 per cent while fragments treated with 2 per cent sodium fluoride solution showed a mean weight loss of 24.97 per cent. Control fragments showed a mean weight loss of 29.39 per cent while fragments treated with the Zin-Ferro-Nitrate solution showed a mean weight loss of 33.59 per cent.

TABLE I

A COMPARATIVE STUDY OF TOOTH DECALCIFICATION UTILIZING ENAMEL-DENTIN FRAGMENTS OF THE SAME TOOTH FOR BOTH EXPERIMENTAL AND CONTROL PURPOSES

GROUP	NUMBER OF TEETH PER GROUP	PERCENTAGE MEAN WEIGHT LOSS OF TREATED AND UNTREATED ENAMEL-DENTIN FRAGMENTS FOLLOWING EXPOSURE TO L. ACIDOPHILUS ORAL STRAIN 12B FOR THIRTY DAYS			
		FRAGMENTS A CONTROL	FRAGMENTS B A.F.*	FRAGMENTS C 2% NAF	FRAGMENTS D Z-F-N†
166	4	29.39	21.08	24.97	33.59
167	4	42.42	30.88	41.35	36.40
168	4	37.70	32.21	26.58	38.53
169	4	43.57	26.77	23.22	37.46
170	4	38.29	27.90	23.72	31.67
171	4	39.73	24.18	26.13	36.03
172	4	36.85	35.09	31.02	26.20
173	4	40.08	25.64	23.37	33.29
174	4	40.79	27.92	28.81	37.27
175	4	40.50	28.69	28.51	40.24
176	4	49.24	29.74	32.21	34.59
177	4	42.69	28.39	32.18	33.96

*A.F. = Acidulated fluoride solution.
†Z-F-N = Zin-Ferro-Nitrate solution.

Table II tabulates a study of thirteen groups composed entirely of untreated fragments. There were no consistent differences in percentage mean weight loss of fragments B and C when compared with fragments A and D (Table I). For example, in Group 178 fragments C showed a mean weight loss of 31.41 per cent while fragments D showed a mean weight loss of 37.40 per cent. In Group 180 fragments C showed a mean weight loss of 32.22 per cent

TABLE II

A COMPARATIVE STUDY OF TOOTH DECALCIFICATION UTILIZING ENAMEL-DENTIN FRAGMENTS OF THE SAME TOOTH AS MEMBERS OF FOUR UNTREATED GROUPS

GROUP	NUMBER OF TEETH PER GROUP	PERCENTAGE MEAN WEIGHT LOSS OF UNTREATED ENAMEL-DENTIN FRAGMENTS FOLLOWING EXPOSURE TO L. ACIDOPHILUS ORAL STRAIN 12B FOR THIRTY DAYS			
		FRAGMENTS A	FRAGMENTS B	FRAGMENTS C	FRAGMENTS D
178	4	35.31	32.45	31.41	37.40
179	4	30.24	29.82	31.85	29.68
180	4	27.38	28.83	32.22	27.92
181	4	30.24	35.99	30.85	30.61
182	4	32.36	34.85	35.57	36.28
183	4	31.78	36.66	30.91	29.20
189	4	36.26	40.67	39.34	32.58
190	4	44.85	38.57	37.39	44.87
191	4	35.73	36.64	39.77	36.10
192	4	37.58	39.36	41.28	40.22
193	4	33.59	39.78	38.66	36.67
194	4	37.29	41.53	38.78	40.47
195	6	34.45	34.50	36.72	36.30

while fragments D showed a mean weight loss of 27.92 per cent. In Groups 179 and 195 very little difference in weight loss was observed among fragments A, B, C, and D.

Table III gives a consolidation of the results concerning the twelve groups numbered 166 to 177 inclusive. Here the untreated fragments showed a mean weight loss of 39.35 per cent. Fragments treated with either an acidulated fluoride solution, a 2 per cent sodium fluoride solution or Zin-Ferro-Nitrate solution showed mean weight losses of 26.88 per cent, 29.73 per cent, and 35.50 per cent respectively. Fragments treated with the acidulated fluoride solution showed a mean reduction in decalcification of 31.69 per cent while fragments treated with either 2 per cent sodium fluoride solution or Zin-Ferro-Nitrate solution showed a reduction in decalcification of 24.45 per cent and 9.79 per cent respectively.

TABLE III

TOOTH DECALCIFICATION IN MATCHED ENAMEL-DENTIN FRAGMENTS OF FORTY-EIGHT TEETH WHICH WERE GIVEN VARIOUS TREATMENTS AND SUBJECTED TO L. ACIDOPHILUS ORAL STRAIN 12B FOR THIRTY DAYS

TYPE OF TREATMENT	ORIGINAL WEIGHT (GM.)	FINAL WEIGHT (GM.)	LOSS		% REDUCTION IN DECALCIFICATION
			WEIGHT	%	
Control	10.1589	6.1615	3.9974	39.35	-
Acidulated fluoride solution	10.1988	7.4574	2.7414	26.88	31.69
2% sodium fluoride solution	9.9688	7.0050	2.9638	29.73	24.45
Zin-Ferro-Nitrate solution	10.1660	6.5574	3.6086	35.50	9.79

Table IV is a consolidation of the results concerning the thirteen groups of untreated fragments numbers 178 to 183 inclusive and 189 to 195 inclusive. Here are shown no significant differences in weight losses between the fragments A, B, C, and D. Results of this control study lend further evidence to the significance of the testing solutions for reducing decalcification of enamel as shown in Table III.

TABLE IV

TOOTH DECALCIFICATION IN MATCHED ENAMEL-DENTIN FRAGMENTS OF FIFTY-FOUR TEETH WHICH RECEIVED NO TREATMENT PRIOR TO EXPOSURE TO L. ACIDOPHILUS ORAL STRAIN 12B FOR THIRTY DAYS

FRAGMENTS	ORIGINAL WEIGHT (GM.)	FINAL WEIGHT (GM.)	LOSS	
			WEIGHT	%
A	10.0685	6.6263	3.4422	34.19
B	10.0978	6.4662	3.6316	35.97
C	10.0862	6.4845	3.6017	35.71
D	10.0890	6.5700	3.5190	34.88

DISCUSSION

In a previous report[1] extracted molar teeth treated with either an acidulated fluoride solution or a 2 per cent sodium fluoride solution showed a similar reduction in decalcification of enamel of approximately 32.50 per cent. In

this investigation the acidulated fluoride solution and the 2 per cent sodium fluoride solution showed a reduction in decalcification of enamel of 31.69 per cent and 24.45 per cent respectively. Such results may be attributed to the fact that in this study the solutions were applied to comparable fragments from the same tooth, while in the former study of whole teeth the solutions were applied to individual teeth. In the study of enamel-dentin fragments the Zin-Ferro-Nitrate solutions reduced decalcification only 9.79 per cent as compared to 11.79 per cent when solutions were applied to whole teeth.[1] The result of this investigation concerning Zin-Ferro-Nitrate solutions confirms the findings of a previous report. This investigation offers further evidence that the latter solutions are far less effective for reducing decalcification of enamel in vitro than either an acidulated fluoride solution or a 2 per cent sodium fluoride solution.

Untreated whole teeth showed a mean weight loss of 11.12 per cent following thirty days' exposure to *L. acidophilus* oral strain 12B.[1] In this study untreated enamel-dentin fragments showed a mean weight loss of 35.19 per cent. Such evidence indicates that the tooth's greatest defense to decalcification exists within the surface layers of the enamel tissue that made up the outer border of the crown.

By two distinct methods it has been shown that the application of either an acidulated fluoride solution or 2 per cent sodium fluoride solution to dental tissue was effective for reducing decalcification of enamel following its exposure to an oral strain of the *L. acidophilus* for thirty days in vitro. However, a question arises: Will the effectiveness of the test solutions be significantly progressive? Studies concerning this phase of the investigation are now in progress.

CONCLUSIONS

Technics utilizing either an acidulated fluoride solution, a 2 per cent sodium fluoride solution, or Zin-Ferro-Nitrate solution reduce decalcification of enamel in vitro 31.69 per cent, 24.45 per cent, and 9.79 per cent respectively.

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AN EVALUATION OF VARIOUS TECHNICS FOR REDUCING DECALCIFICATION OF ENAMEL

III. A Progressive Study of Decalcification

HUBERT B. PALMER, SUSIE OVERSTREET, B.S., AND MARILYN SACKS, B.S.

Department of Dental Research, Dental Division, Army Medical Department Research and Graduate School, Army Medical Center, Washington, D. C.

PALMER, Overstreet, and Sacks[1, 2] have shown that when the enamel surfaces of whole teeth and of enamel-dentin fragments were treated with either an acidulated fluoride solution, a 2 per cent sodium fluoride solution, or a Zin-Ferro-Nitrate solution and immersed in broth seeded with lactobacilli, decalcification of enamel was reduced.

Cheyne,[3] Bibby,[4] Knutson and Armstrong,[5, 6, 7] Knutson, Armstrong, and Feldman,[8] and Jordan, Wood, Allison, and Irvin[9] have reported a decrease in the incidence of dental caries in groups of individuals following the employment of a method utilizing certain fluoride solutions. Arnold, Dean, and Singleton[10] and Stone, Lawton, Bransby, and Hartley[11] have reported no significant reduction in the incidence of dental caries for groups of individuals whose teeth had been treated with an acidulated fluoride solution. Crawford,[12] Younger,[13] and Gillard, Boelsche, and Summers[14] have reported a decrease in the incidence of dental caries in groups of patients following the employment of a method utilizing various pharmaceuticals in combination. Likins and McClure[15] in their studies of powdered enamel treated with various fluoride solutions suggest that the adsorbed fluoride is removed from the enamel by the washing process. Scott, Picard and Wyckoff[16] have reported that the CaF_2 deposits formed upon slabs of enamel during their immersion in a fluoride solution can be removed by washing in water.

The purpose of this investigation has been twofold: (a) To determine the effectiveness that such test solutions as applied in previous studies[1, 2] may demonstrate for reducing decalcification* of enamel when both treated whole teeth and dental fragments were exposed to *Lactobacillus acidophilus* for prolonged periods. (b) To determine if 2 per cent sodium fluoride solution was equally effective for reducing decalcification of enamel in both decalcified and nondecalcified dental tissues when it was applied to both whole teeth and enamel-dentin fragments.

METHOD OF STUDY

Extracted, erupted, noncarious whole third molar teeth, representing one hundred adults, were thoroughly cleaned with scalers, rubber cups, and pumice. They were then dried at 42° C. for twenty-four hours, after which the teeth were

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*Decalcification of dental tissue as referred to in this report is based upon the difference noted between the original and final weight of either the whole tooth or of the enamel-dentin fragment following exposure to the lactobacilli.

weighed and divided into five groups. Thirty-two teeth (group 1) were exposed to *L. acidophilus** for thirty days before treatment. After this the teeth were reweighed; the roots of the teeth were covered with paraffin and their crowns immersed in individual test tubes containing 5 c.c. of 2 per cent sodium fluoride solution for thirty-five minutes. Following the removal of the paraffin they were immersed in 70 per cent ethyl alcohol for twenty minutes and then re-exposed to *L. acidophilus* for seventy days. In groups 2, 3, and 4, half the crown and the entire root portion of each tooth were first covered with paraffin. The exposed half of each crown in groups 2, 3, and 4 (fifteen, fifteen, and twenty-two teeth respectively) was treated with test solutions according to the method described in a previous report.[1] Teeth in group 2 received an acidulated fluoride solution; those in group 3 received a 2 per cent sodium fluoride solution, while those in group 4 were treated with the Zin-Ferro-Nitrate solution. Following the treatment of all the teeth in groups 2, 3, and 4, the paraffin covering on each tooth was removed. Teeth in Group 5, which included twenty-one teeth in number, were untreated and served as controls. Teeth in groups 2, 3, 4, and 5 were immersed in 70 per cent ethyl alcohol for twenty minutes and then subjected to *L. acidophilus* for one hundred days according to the technique described previously.[1] All the teeth of the five groups at intervals of 30, 70, and 100 days following exposure to *L. acidophilus* were washed clean, dried at 42° C. for twenty-four hours, and then weighed. Following the 30- and 70-day periods, the teeth were immersed in 70 per cent ethyl alcohol for twenty minutes before being resubjected to *L. acidophilus*.

One hundred and two noncarious molar teeth, extracted from eighty-one adults, were prepared as follows. They were thoroughly cleaned with scalers, rubber cups, and pumice. Using separating disks the crowns of forty-eight teeth, after being removed from the root at the cemento-enamel junction, were cut into four similar parts known as fragments A, B, C, and D. A single enamel-dentin fragment known as A^1 similar to the preceding fragment A was removed from each of fifty-four teeth. (A previous study[2] has shown no significant difference between untreated matched enamel-dentin fragments.) The fragments were washed for a few seconds in running tap water in order to remove any dust or foreign particles present. The 246 fragments were then dried at 42° C. for six hours, after which they were weighed and divided into five groups. Group 6 was represented by fifty-four A^1 fragments which were exposed to *L. acidophilus* for thirty days before treatment. At the end of the thirty-day period, after the fragments had been reweighed, they were immersed in individual test tubes containing 5 c.c. of 2 per cent sodium fluoride solution for thirty-five minutes. They were then immersed in 70 per cent ethyl alcohol for twenty minutes after which they were re-exposed to *L. acidophilus* for thirty-four days. Groups 7, 8, 9, and 10 were each represented by forty-eight fragments. Fragments B, C, and D for the respective groups 7, 8, and 9 had their cut enamel surfaces and exposed dentin surfaces covered with paraffin after which they were treated with a test solution according to method described in

L. acidophilus oral strain 12B has been used throughout the experiment.

a previous report.[2] The exposed (unwaxed) enamel surfaces of fragments B were treated with an acidulated fluoride solution, while similar surfaces of fragments C were treated with the 2 per cent sodium fluoride solution. The exposed surfaces of fragments D were treated with the Zin-Ferro-Nitrate solution. Following the treatment of all the fragments in groups 7, 8, and 9, the paraffin covering on each fragment was removed. Group 10, composed of 48 A fragments, was untreated and served as controls. Fragments of groups 7, 8, 9, and 10 were immersed in 70 per cent ethyl alcohol for twenty minutes and subjected to *L. acidophilus* for sixty-four days according to the technique described previously.[2] All the fragments, at intervals of 30, 47, and 64 days, following exposure to *L. acidophilus* were washed clean, dried at 42° C. for six hours, and then weighed. Following the 30- and 47-day exposure period, the fragments were immersed in 70 per cent ethyl alcohol for twenty minutes before being resubjected to *L. acidophilus*.

RESULTS

In Table I are shown the percentage mean weight losses of treated and untreated whole teeth following exposure to *L. acidophilus* for periods of 30, 70, and 100 days. Teeth in group 1 showed a mean weight loss of 19.92 per cent after an initial exposure to *L. acidophilus* for thirty days followed by treatment with 2 per cent sodium fluoride solution and re-exposure to the *L. acidophilus* for 70 more days. For evaluating the effectiveness of the 2 per cent sodium fluoride solution upon previously decalcified teeth as in group 1, one must compare the mean weight loss with the 28.87 per cent mean weight loss of group 5 (composed originally of nondecalcified teeth which were untreated), after exposure of group 5 to *L. acidophilus* for 100 days. Though the initial mean weight loss for the teeth of group 1 was 10.42 per cent after 30 days of exposure to *L. acidophilus* compared to the 11.38 per cent loss for those of group 5 for the same period, further study of Table I shows that 2 per cent sodium fluoride solution was most effective for reducing decalcification in previously decalcified teeth. The teeth in group 1 following their treatment with 2 per cent sodium fluoride solution showed a mean weight loss of 9.50 per cent (19.92 per cent minus 10.42 per cent) during the 30- to 100-day exposure period to *L. acidophilus*. The teeth of group 5 showed a mean weight loss of 17.49 per cent (28.87 per cent minus 11.38 per cent) during the 30- to 100-day exposure period. Thus the application of 2 per cent sodium fluoride solution to previously decalcified teeth reduced further decalcification of enamel 45.69 per cent. Notwithstanding the fact that the 2 per cent sodium fluoride solution was applied to the whole crown of each tooth in group 1, the 45.69 per cent reduction in decalcification compares favorably with the 23.86 per cent (Table II) reduction in decalcification of enamel for group 3, where half the crown of previously nondecalcified teeth was treated with 2 per cent sodium fluoride solution and exposed to *L. acidophilus* for 70 days.

In Table I the previously nondecalcified teeth treated with the acidulated fluoride solution in group 2 consistently demonstrated after each period of

exposure to *L. acidophilus* less weight loss than the previously nondecalcified teeth of groups 3 and 4, which were treated with 2 per cent sodium fluoride solution and Zin-Ferro-Nitrate solution respectively.

TABLE I

A STUDY OF THE PERCENTAGE MEAN WEIGHT LOSS OF TREATED AND UNTREATED WHOLE THIRD MOLAR TEETH FOLLOWING THEIR EXPOSURE TO L. ACIDOPHILUS ORAL STRAIN 12 B

GROUP	TREATMENT	NUMBER OF TEETH	TOTAL WEIGHT OF GROUPS IN GRAMS BEFORE AND AFTER EXPOSURE TO THE BACTERIA FOR DAYS SHOWN WITH PERCENTAGE MEAN WEIGHT LOSS			
			ORIGINAL	30	70	100
1	Subjected to L.a.* then 2% sodium fluoride solution	32	56.6491	50.7516	47.6324	45.3661
			% loss:	10.42	15.92	19.92
2	Acidulated fluoride solution	15	26.9065	25.0525	22.5125	20.9210
			% loss:	6.90	16.34	22.25
3	2% Sodium fluoride solution	15	26.4638	24.5618	21.8523	20.3757
			% loss:	7.19	17.43	23.01
4	Zin-Ferro-Nitrate solution	22	39.1185	35.5805	30.8181	28.3483
			% loss:	9.05	21.22	27.54
5	Controls	21	37.1172	32.8958	28.6243	26.4032
			% loss:	11.38	22.89	28.87

*Exposed to *Lactobacillus acidophilus* for 30 days at 37° C., then treated with 2 per cent NaF solution.

In Table II are shown the percentage reductions in decalcification of enamel that were achieved by various methods of treatment for each period that the teeth were exposed to *L. acidophilus*. The previously decalcified teeth treated with the 2 per cent sodium fluoride solution showed reductions in decalcification of 30.45 per cent for 70 days' exposure and 31.01 per cent after 100 days' exposure. All three of the test solutions, namely the acidulated fluoride solution, the 2 per cent sodium fluoride solution, and the Zin-Ferro-Nitrate solution, as applied to previously nondecalcified teeth, demonstrated a diminishing reduction in decalcification of enamel following each period the teeth were exposed to *L. acidophilus*. For example, those teeth treated with the Zin-Ferro-Nitrate solution showed a 20.48 per cent reduction in decalcification of enamel after 30 days of exposure to *L. acidophilus*. Such teeth showed a 7.30 per cent reduction in decalcification of enamel after 70 days of exposure and only 4.61 per cent reduction in decalcification of enamel after 100 days' exposure.

TABLE II

A PROGRESSIVE STUDY OF THE EFFICACY OF VARIOUS AGENTS FOR REDUCING DECALCIFICATION OF ENAMEL OF WHOLE TEETH EXPOSED TO L. ACIDOPHILUS ORAL STRAIN 12 B

TREATMENT	NUMBER OF TEETH	PERCENTAGE REDUCTION IN DECALCIFICATION OF GROUPS COMPARED TO CONTROL TEETH EXPOSED TO L.A. FOR PERIOD OF DAYS SHOWN		
		30	70	100
Subjected to L.a. before treating with 2% sodium fluoride solution	32	—	30.45	31.01
Acidulated fluoride solution	15	39.37	28.62	22.94
2% Sodium fluoride solution	15	36.82	23.86	20.30
Zin-Ferro-Nitrate solution	22	20.48	7.30	4.61

In Table III are shown the percentage mean weight losses of treated and untreated enamel-dentin fragments following their exposure to *L. acidophilus* for periods of 30, 47, and 64 days. Fragments in group 6 showed a mean weight loss of 46.21 per cent after an initial exposure to *L. acidophilus* for 30 days followed by treatment with 2 per cent sodium fluoride solution and re-exposed to *L. acidophilus* for 34 more days. For evaluating the effectiveness of the 2 per cent sodium fluoride solution upon previously decalcified enamel-dentin fragments as applied in group 6, one must compare the mean weight loss with the 67.59 per cent mean weight loss of group 10 (composed originally of non-

TABLE III

A STUDY OF THE PERCENTAGE MEAN WEIGHT LOSS OF TREATED AND UNTREATED MATCHED ENAMEL-DENTIN FRAGMENTS FOLLOWING THEIR EXPOSURE TO L. ACIDOPHILUS ORAL STRAIN 12 B

GROUP	TREATMENT	FRAGMENTS NUMBER	FRAGMENTS TYPE	TOTAL WEIGHT OF GROUPS IN GRAMS BEFORE AND AFTER EXPOSURE TO BACTERIA FOR DAYS SHOWN WITH PERCENTAGE MEAN WEIGHT LOSS ORIGINAL	30	47	64
6	Subjected to L.a. before treating with 2% sodium fluoride solution	54	A'*	10.0685	6.6263	6.0929	5.4164
				% loss:	34.19	39.49	46.21
7	Acidulated fluoride solution	48	B	10.1988	7.4574	6.2881	4.9151
				% loss:	26.88	38.35	51.81
8	2% Sodium fluoride solution	48	C	9.9688	7.0050	6.0032	4.6838
				% loss:	29.73	39.79	53.02
9	Zin-Ferro-Nitrate solution	48	D	10.1166	6.5574	5.0083	3.5846
				% loss:	35.50	49.71	64.59
10	Controls	48	A	10.1593	6.1615	4.7120	3.2929
				% loss:	39.35	53.62	67.59

*Fragments A' represent 54 teeth while fragments A, B, C, D represent 48 different teeth.

decalcified enamel-dentin fragments which were untreated) after exposure to *L. acidophilus* for 64 days. Though the initial mean weight loss for the enamel-dentin fragments of group 6 was 34.19 per cent after 30 days' exposure to *L. acidophilus* as compared to the 39.35 per cent loss for those of group 10 for the same period, further study of Table III shows that 2 per cent sodium fluoride solution was most effective for reducing decalcification in previously decalcified enamel-dentin fragments. The fragments in group 6 following treatment with 2 per cent sodium fluoride solution showed a mean weight loss of 12.02 per cent (46.21 per cent minus 34.19 per cent) during the 30- to 64-day exposure period to *L. acidophilus*. The fragments of group 10 showed a mean weight loss of 28.24 per cent (67.59 per cent minus 39.35 per cent) during the 30- to 64-day exposure period. Thus the application of 2 per cent sodium fluoride solution to previously decalcified fragments reduced further decalcification of enamel and dentin 57.44 per cent. Notwithstanding the fact that the 2 per cent sodium fluoride was applied both to enamel and dentin in group 6, the 57.44 per cent reduction in decalcification compares more than equally well with the 24.45 per cent (Table IV) reduction in decalcification of enamel for group 8, where only the enamel of the fragments was treated with the 2 per cent sodium fluoride solution and exposed to *L. acidophilus* for 30 days.

In Table III the previously nondecalcified fragments treated with the acidulated fluoride solution in group 7 consistently demonstrated after each period of exposure to *L. acidophilus* less weight loss than the previously nondecalcified teeth of groups 8 and 9 which were treated with 2 per cent sodium fluoride solution and Zin-Ferro-Nitrate solution respectively.

In Table IV are shown the percentage reductions in decalcification of enamel that were achieved by various methods of treatment for each period that the fragments were exposed to *L. acidophilus*. The previously decalcified fragments treated with the 2 per cent sodium fluoride solution showed reductions in decalcification of 26.36 per cent for 47 days' exposure and 31.64 per cent for 64 days' exposure. All three of the test solutions—namely, the acidulated fluoride solution, the 2 per cent sodium fluoride solution, and the Zin-Ferro-Nitrate solution—as applied to previously nondecalcified fragments demonstrated a diminishing reduction in decalcification of enamel following each

TABLE IV

A PROGRESSIVE STUDY OF THE EFFICACY OF VARIOUS AGENTS FOR REDUCING DECALCIFICATION IN MATCHED ENAMEL-DENTIN FRAGMENTS FOLLOWING THEIR EXPOSURE TO L. ACIDOPHILUS ORAL STRAIN 12 B

TREATMENT	NUMBER OF FRAGMENTS	PERCENTAGE REDUCTION IN DECALCIFICATION OF GROUPS COMPARED TO CONTROL FRAGMENTS EXPOSED TO L.A. FOR PERIOD OF DAYS SHOWN		
		30	47	64
Subjected to L.a. before treating with 2% sodium fluoride solution	54	—	26.36	31.64
Acidulated fluoride solution	48	31.69	28.48	23.35
2% Sodium fluoride solution	48	24.45	25.80	21.56
Zin-Ferro-Nitrate solution	48	9.79	7.30	4.47

period the fragments were exposed to *L. acidophilus*. For example, those fragments treated with the acidulated fluoride solution showed a 31.69 per cent reduction in decalcification of enamel after 30 days' exposure to *L. acidophilus*. Such fragments showed a 28.48 per cent reduction in decalcification of enamel after 47 days' exposure and only 23.35 per cent reduction in decalcification of enamel after 64 days' exposure.

DISCUSSION

In previous reports[1, 2] the authors reported that the application of either an acidulated fluoride solution, a 2 per cent sodium fluoride solution, or Zin-Ferro-Nitrate solution to whole teeth or enamel-dentin fragments followed by 30 days' exposure to *L. acidophilus* showed that the test solutions were less effective in reducing decalcification of enamel than has been recorded in Table II of this report. Such differences may be attributed to the variations of teeth in resisting decalcification and the use of a smaller sample in this study. Nevertheless, the three reports have consistently shown that the acidulated fluoride solution as applied to nondecalcified teeth or fragments was the most satis-

factory for reducing decalcification of enamel, that 2 per cent sodium fluoride solution was second best, and that the Zin-Ferro-Nitrate solution was the least satisfactory.

The application of the test solutions to both whole teeth and enamel-dentin fragments has been most advantageous. Such a method increased the tooth sampling, limited those variations due to chance, and permitted a dual analysis as proof for each experiment. Findings will have greater significance as they have been based upon an abundance of evidence.

The acidulated fluoride solution, the 2 per cent sodium fluoride solution, and the Zin-Ferro-Nitrate solution as applied to previously nondecalcified whole teeth and fragments demonstrated a diminishing reduction in decalcification of enamel when the teeth and fragments were under prolonged exposure to *L. acidophilus*. Evidently the protective chemical combination of the testing solution and the dental tissue for reducing decalcification has been undergoing gradual loss or destruction. In the light of such evidence one does question the mechanism by which topical solutions render a tooth protection against dental caries when they are applied to teeth of individuals in accordance with those technics recommended by Knutson[17] and Gottlieb.[18] Perhaps, however, the role of topical solutions for controlling dental caries may succeed itself in importance as a useful adjunct in the application of methods for studying the etiology of dental caries.

The application of the 2 per cent sodium fluoride solution to both decalcified teeth and enamel-dentin fragments reduced further decalcification of enamel as effectively as when applied either to nondecalcified teeth or to nondecalcified enamel-dentin fragments. Variations in the technic of applying alkaline and acidulated fluoride solutions to teeth appear to be promising for controlling decalcification of enamel. Studies concerning this phase of the investigation are in progress.

CONCLUSIONS

1. The acidulated fluoride solution, the 2 per cent sodium fluoride solution, and the Zin-Ferro-Nitrate solution as applied both to nondecalcified teeth and enamel-dentin fragments, became progressively less effective for reducing decalcification of enamel when the teeth or fragments were under prolonged exposure to *L. acidophilus*.

2. The application of the 2 per cent sodium fluoride solution to both decalcified teeth and enamel-dentin fragments reduced further decalcification of enamel as effectively as when applied to either nondecalcified teeth or to nondecalcified enamel-dentin fragments.

The authors wish to express their sincere appreciation to all the dentists in military and civilian life who have contributed so graciously in furnishing us with teeth and necessary data for this investigation.

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THE TOXICITY OF IODOACETIC ACID AND ITS QUANTITATIVE RELATIONS TO INHIBITION OF GLYCOLYSIS AND DENTAL CARIES

CLAES LUNDQVIST, L.D.S., D.D.S., M.L.

From the Department of Pharmacology, University of Lund, and the Department of Oral Surgery, State Dental School, Malmö, Sweden

THE enzymatic breakdown of carbohydrates in the diet to acid products in direct contact with the tooth is an essential pathogenic stage in the mechanism of beginning caries. Consequently, the question is raised as to the possibilities of inhibiting this chain of reactions through local application of drugs either through direct application or by mixture with the diet.

The glycolysis on a tooth surface follows essentially the same course as the enzymatic action on carbohydrates and their intermediary products in the digestive apparatus. Consequently, inhibition of the oral enzyme activity can also produce a nondesirable toxic effect in the function of the organism.[12] This investigation is an experimental study on rats, attempting to clarify the quantitative data required for a determination of dosage and effects.

The specific enzyme inhibiting effect of iodoacetic acid (I-ac) has been employed in the study of glycolysis according to Meyerhof's[18] system and, for example, employed experimentally in investigations of the intermediary carbohydrate metabolism in muscle by Lundsgaard[15, 16] and others. In view of the fact that the caries process implies an enzymatic breakdown of carbohydrates on the tooth surface, Miller[19] concluded that the inhibiting effect of I-ac (and fluorides) would influence dental caries. In his experiment on rats with the administration of the afore-mentioned drugs in both food and water, a marked caries inhibition was noted. McClure and Arnold[17] repeated these experiments on a larger scale and clearly confirmed the conclusions drawn by Miller. Dale and Keyes[4] demonstrated the same inhibitory effect on the caries process in the Syrian hamster.

Dale and Powell[3] obtained a comparatively greater inhibitory effect in their rat experiment when administering I-ac in the food than when administering it with the drinking water. The same authors[20] demonstrated the caries-inhibitory effect by parenteral administration of sodium iodoacetate. In experiments by Dale[5] with parenteral administration in desalivated rats, some inhibition was noted, yet not enough to fully explain an endogenous effect via the tooth's own nutrition. The investigation by Thomassen and Leicester[21] with radioactive iodine in the I-ac seems, however, to support the opinion that

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I-ac acts not only directly through food contact with the tooth surface, but also that the I-ac reaches the pulp through the blood, and the tooth surface via the salivary route.

In previous experiments, I-ac was added to the food to a concentration of 0.02 per cent. The rate of growth was normal and no toxic symptoms were reported. This is in contrast to results of rat experiments by Laszt and Verzar[14] on the growth-inhibitory effect of I-ac in the same concentration. Their experimental animals exhibited marked toxic changes.

No attempts to determine the minimum dose inhibiting caries have been reported and the toxic effect by oral administration has not been determined quantitatively.

In developing and evaluating caries-inhibiting drugs which interfere with the enzymatic breakdown of carbohydrates, data regarding the minimum active dose and the toxicity of the drugs are necessary. A concurring comparison between the inhibitory effect in vitro and in vivo provides a basis for the relationship between the two tests, giving a lead for determination of dosage in experimental caries investigations in animals.

I-ac was chosen as typical of enzyme inhibitors, because of its proved ability to inhibit caries activity and its toxic damage when administered in large doses. Since I-ac is not a stable compound, freshly prepared solutions were used in this study.

The purpose of this investigation is:

1. To make comparative study of the effect in vitro and in vivo of I-ac as a caries-inhibiting drug by oral administration in rats.

2. To determine the safety margin of I-ac in oral administration (lethal dose to minimum caries-inhibiting dose).

3. To determine possible toxic effect upon the organism by an active caries-inhibitory dose.

Experimental

In vitro experiments

I-ac Inhibition.—The calcium and phosphorus concentration in samples consisting of saliva from caries active persons + glucose + powdered enamel is increased by incubation at 37° C. for 4 hours (Fosdick,[7] Abdon and Lundqvist[1] and others). According to Fosdick and Calandra[8] this test can be used to test enzyme-inhibiting agents which can be added in different concentrations.

The calcium concentration was determined according to the method of Kramer and Tisdall[13] in duplicate after the incubation period during which the sample was constantly shaken.

Nonneutralized I-ac gives, in higher concentrations, an acid effect by dissolving calcium from the powdered enamel. The inhibitory effect is thus concealed in high concentrations, but is present at lower concentrations which are of interest for the experiment.

Results: Fig. 1 shows the complete inhibition of the enzyme activity with I-ac concentrations from 0.05 to 0.0005 per cent with decreasing effect below 0.00025 per cent. The results can be almost identically reproduced in samples with varying "saliva activity."

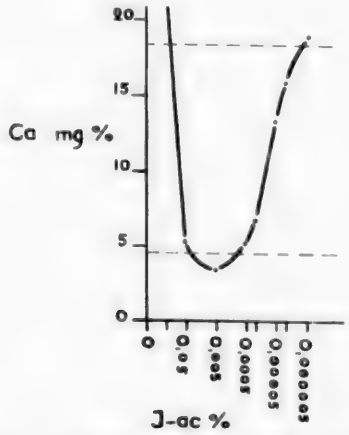


Fig. 1.—The effect of I-ac in saliva glucose enamel test.

ANIMAL EXPERIMENTS

I-ac Toxicity.—From the reports by Hall and Field[10] regarding the lethal dose of I-ac by parenteral administration in albino rats, LD_{50} is calculated to be about 60 mg. per kilogram body weight. After oral administration of a lethal dose the animal dies without typical rigor, indicating a different biologic mechanism. To obtain a basis for the caries experiments in the rat a toxicity determination was made through oral administration of I-ac. Approximately 1 ml. of I-ac brought to pH 7 with NaOH was administered through a stomach tube. The animals were not starved. Two hundred and twenty rats were used, 20 for each concentration.

Results: The surviving animals exhibited reduced activity during the first day, but acted normally on the next day. With a few exceptions, the remaining rats died within 24 hours. The death was preceded by singular convulsions without permanent muscle rigidity. The distribution is shown in Table I.

From Table I, LD_{50} is calculated according to the method of Bliss[2] to be 116 ± 13 mg. per kilogram body weight, the lethal doses varying from about 50 to 190 mg. per kilogram body weight.

I-ac Elimination.—For the purpose of orientation regarding possible accumulative effect of I-ac, a high nonlethal dose was administered orally for a series of days. Ten rats, each weighing around 60 grams, were fed 33.3 mg. per kilogram body weight I-ac for 15 days, after which the experiment was terminated.

TABLE I

TOXICITY OF PERORAL ADMINISTRATION OF IODOACETIC ACID. ANIMAL: ALBINO RAT; MEAN WEIGHT, 60 GRAMS

DOSE (MG. PER KG. BODY WEIGHT)	NUMBER DEAD (TOTAL)	DEATH RATE (%)
33.3	0/20	0
50.0	0/20	0
66.7	0/20	0
83.3	4/20	20
100.0	8/20	40
116.7	7/20	35
133.3	13/20	65
150.0	15/20	75
166.7	18/20	90
183.3	19/20	95
200.0	19/20	95

Results: One animal died on the last day. At this time all animals had diarrhea. An accumulative effect is not evident, since this tentative experiment suggests rapid elimination with effect on the bowel function.

I-ac Caries Inhibition.—

Preparation of Food: The food was composed principally according to the formula of Hoppert, Webber and Canniff[11] (HWC), the size of particles being controlled.

TABLE II

COMPOSITION OF FOOD IN I-AC EXPERIMENT

FOOD INGREDIENTS	PER CENT
Corn	50
Dried milk	30
Sucrose	10
Linseed meal	6
Alfalfa meal	3
Na Cl	1
	100

I-ac has been added so that every corn particle should be coated with a layer of I-ac. For that reason the corn was immersed in a solution of I-ac in 108 mesh cloth, and thereafter dried to its original weight according to the following method: the amount of fluid retained by a given amount of corn was determined after soaking for 2 minutes and then wringing in the cloth. By this method the I-ac concentration required to give an end product which would give the proposed I-ac concentration in the food after the remaining food ingredients had been added was determined. The corn for the control group was treated in the same manner with water.

The I-ac stability in the corn was determined by in vitro experiments as described previously by adding water-treated and I-ac-treated corn respectively. Samples were tested after 1, 3, 5, 7, 14, and 18 days. No reduction in activity was noted, and the effect corresponded with the previously established values with known I-ac concentration. In accordance with this, new food was prepared every 8 to 10 days during the experimental period. The control

demonstrated that the caries-inhibiting diet contained stable I-ac in the proposed concentrations.

Test Groups: The experiment was made with albino rats of uniform stock. One hundred and fourteen 3- to 4-week-old rats were selected from time to time for experimental animals and distributed 2 to 4 animals to a cage. The animals were evenly distributed in regard to litter and sex. The following test groups were arranged:

HWC (Control group)
HWC + I-ac 0.0002 per cent
HWC + I-ac 0.002 per cent
HWC + I-ac 0.02 per cent

Experimental Period: The experimental period lasted 100 days. The food was weighed out daily. Significant losses were avoided by placing a funnel over the food container with a loose perforated disk covering the food as suggested by Farris.[6] All animals were weighed once or twice each week. Water was given ad libitum (fluorine content, 0.2 to 0.4 mg. per liter).

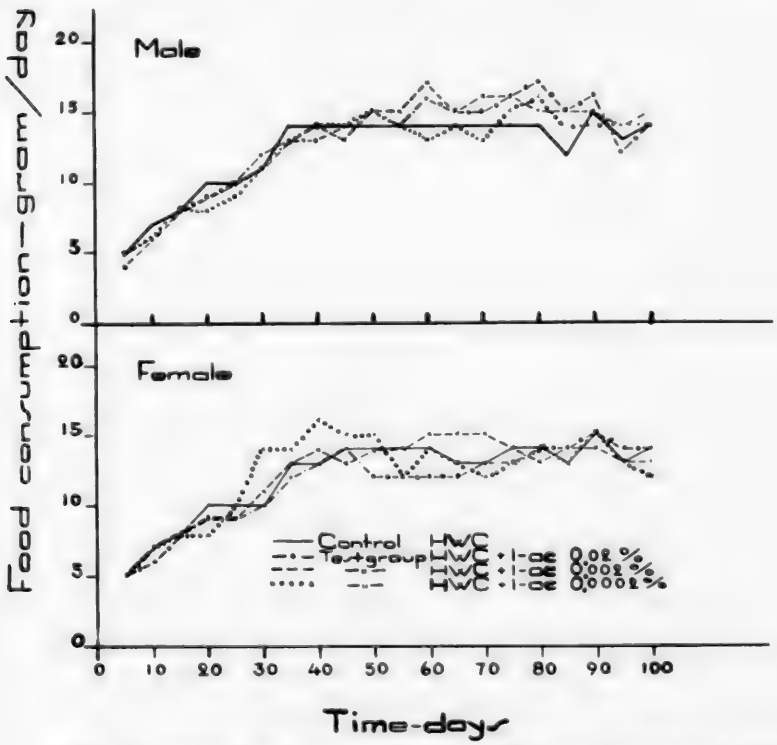


Fig. 2.—Average food consumption per animal during experimental period.

Figs. 2 and 3 show the food intake and weight increase, which give evidence of no differences between the test groups and that all animals showed normal development.

Results:

General observation: Toward the end of the experiment, red blood cell counts were made and hemoglobin was determined. There was no difference between the groups.

At the end of the period, the animals were killed with ether and immediately autopsied. Macroscopically, the experimental animals exhibited no pathologic changes. Although according to Laszt and Verzar[14] the adrenal glands increase in weight in l-ac poisoning, the left adrenals were weighed without finding significant variations. In a selection from each group, the livers and kidneys were examined histologically. Neither in hematoxylin-eosin or sudan III stains could any pathologic changes be observed.

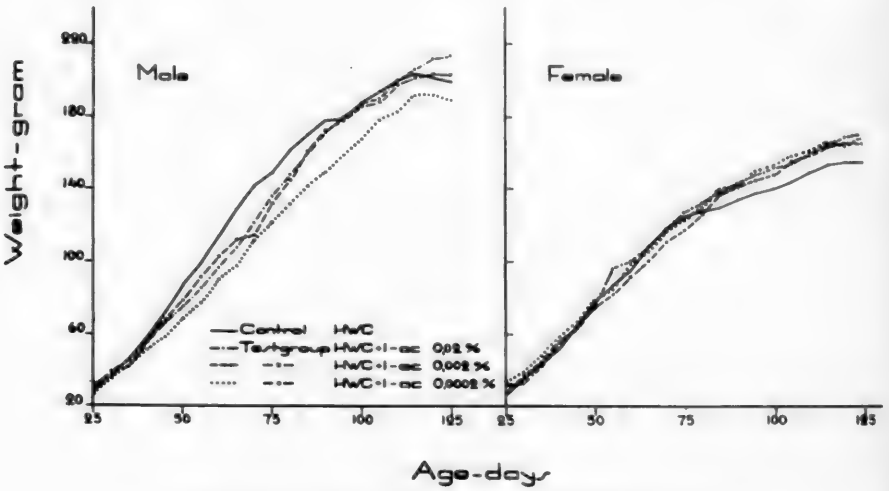
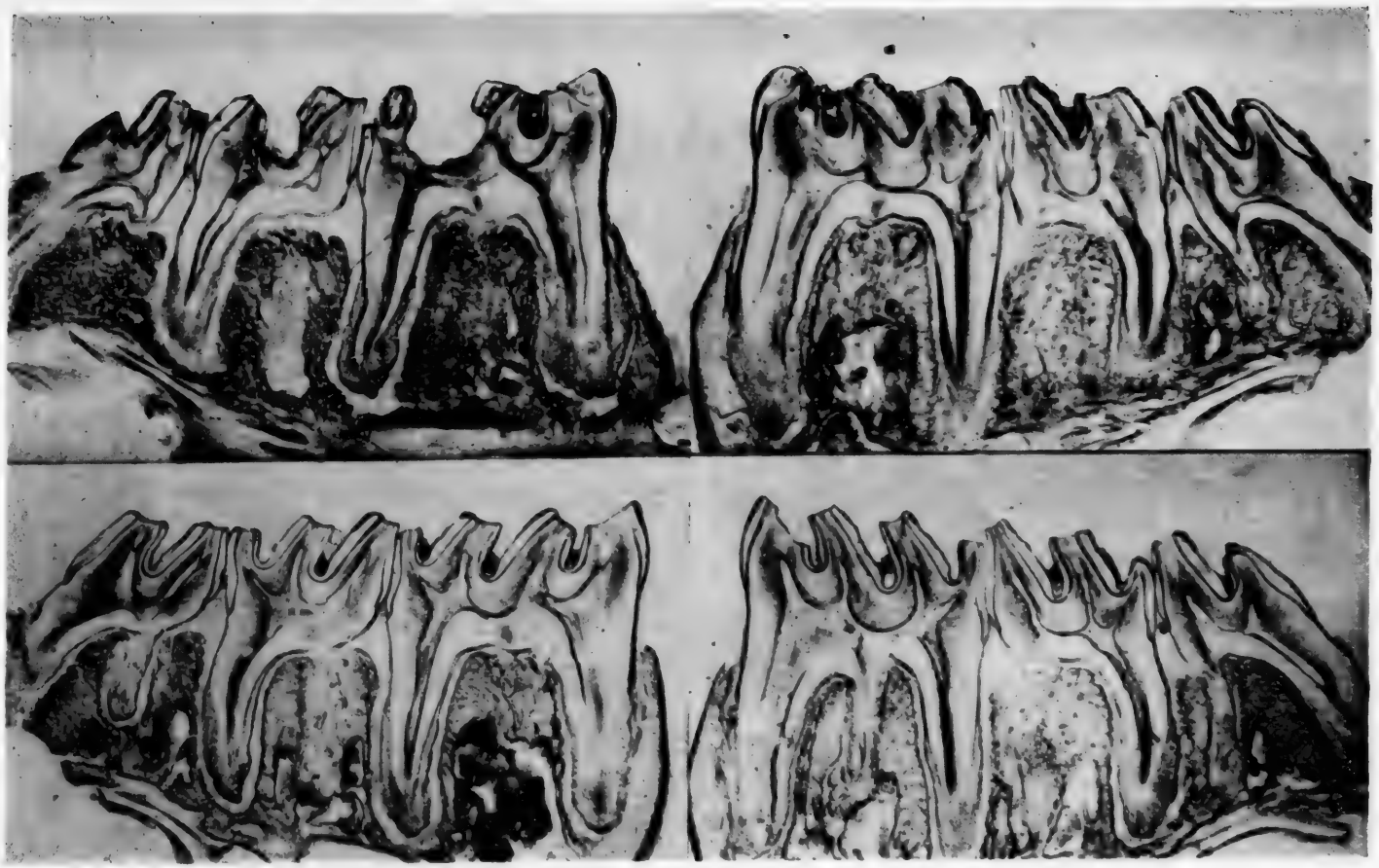


Fig. 3.—Weight increase during experimental period.

Teeth: After cleaning and drying, all lower molars were examined under a dissecting microscope. The diagnosis of caries was based on cusp destruction with softened dentin and undermining fissure caries. Marked differences were observed between the test groups. There are great difficulties in determining the fissures, therefore the possibilities of getting a complete view of the caries are limited in this examination. Definite caries diagnosis was obtained by examining ground sections (Fig. 4). These were made by preparing the lower left quadrant in all experimental animals according to the method of Gustafsson.[9] Each quadrant was ground buccally and lingually to the least possible thickness, carefully confining the section to the midline of the teeth through the center of the pulp of the second molar. The section was then microscopically examined in transmitted light.

The ground section permits a distinct classification of hard tissue defects on the teeth and it has been possible to differentiate between simple fracture, cusp caries with different degree of destruction, and, most significant, fissure caries. Enamel cracks often occur in the bottoms of the fissures, as well as



C. D.

Fig. 4. Ground sections from different experimental groups. Advanced fissure caries in control group and two initial fissure cavities in first molar in 1-ac 0.0002 per cent group. No caries in other groups. *A*, Control. *B*, 1-ac 0.0002 per cent. *C*, 1-ac 0.002 per cent. *D*, 1-ac 0.02 per cent.

TABLE III

MICROSCOPIC EXAMINATION OF GROUND SECTIONS

TEST GROUPS	NUMBER OF EXAMINED ANIMALS	NUMBER OF EXAMINED TEETH	FRACTURED CUSPS WITHOUT CARIES (%)†	CARIES					
				TOTALLY FRACTURED CUSPS WITH CARIES (%)	PARTIALLY FRACTURED CUSPS WITH CARIES (%)	NONFRACTURED CUSPS WITH CARIES (%)	FISSURE CARIES CAVITIES (%)	CARIOUS TEETH (%)	NUMBER OF CARIOUS ANIMALS
Control	26*	78	4	36	9	1	35	36	19
I-ac 0.0002 %	30	90	7	9	3	4	30	29	20
I-ac 0.002 %	30	87	12	4	8	4	6	15	9
I-ac 0.02 %	27	79	6	3	3	1	3	5	2

*One jaw destroyed at preparation.

†All percentage values calculated on number of teeth.

cusp fractures without discoloration. Bacterial invasions were differentiated from the typical carious defects of the dentin which usually undermine the enamel.

Table III shows distribution of the different defects and their occurrence in the test groups. The even distribution of fractured cusps, indicating a uniform influence within the groups from the mechanical resistance offered by the food during mastication, may be observed. This was a specific purpose in preparation of the experimental food.

The predominant caries defects consist of totally fractured cusps with extensive caries of the dentin and caries at the bottom of the fissures. Both these types of defect show the same tendency, with highest frequency in the control group. While there is marked reduction in the occurrence of totally fractured, carious cusps in all I-ac groups, this inhibition is most clearly exhibited in the fissure caries at an I-ac concentration of 0.02 per cent and 0.002 per cent. The inhibitory effect is negligible at the lowest concentration, 0.0002 per cent. Approximal caries was not found.

DISCUSSION

From the in vitro experiments it is evident that the saliva-glucose-enamel test, in the form employed, has proved especially suitable for testing a glycolysis-inhibiting drug. The regularity with which the experimental results can be reproduced supports this belief. A rapid method for calcium or phosphorus determination would render the test still more practical.

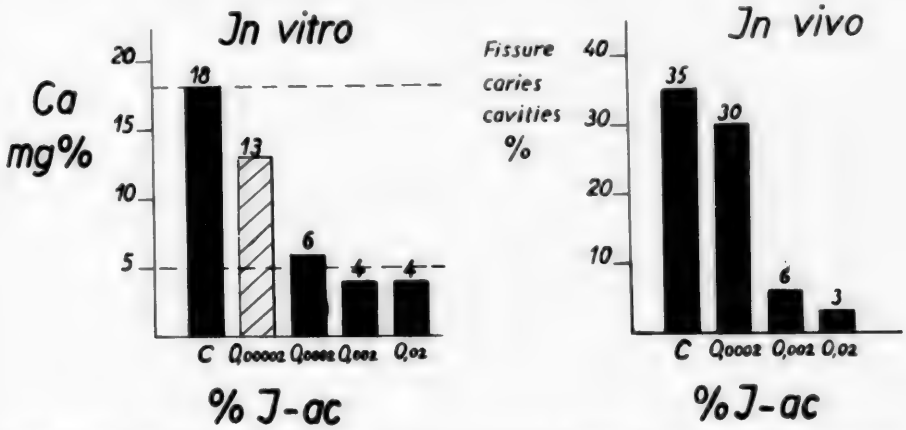


Fig. 5.—The effect of I-ac in vitro and in vivo as a caries inhibitor. Diagrams for comparison.

I-ac gives a considerable inhibiting effect in the saliva tests. The toxicity test demonstrated the earlier well-known biologic ill effects of large doses.

These results demonstrate that I-ac fulfills the requirements of a glycolysis inhibitor. In addition, the experiment clearly verifies earlier work demonstrating the caries-inhibiting effect of I-ac in animals.

Of primary interest is the comparison between the quantitative data obtained from in vitro experiments on one side and the caries experiments in rats

on the other. The results are best shown by Fig. 5. The in vitro diagram has been calculated for the I-ac concentrations used in the animal experiments. The caries incidence has been derived from the occurrence of fissure caries. A comparison between the two diagrams does not necessarily indicate an analogous mechanism in the two types of experiments, but the in vitro experiment serves as a practical indicator of the outcome of animal experiments. From this viewpoint the in vitro experiment proved itself 10 times more sensitive than the animal experiment. The result of this comparison, however, will differ, depending upon which calculation the caries incidence is based on (Table III). A marked caries inhibition occurs with the I-ac concentration of 0.002 per cent, which is a concentration 10 times lower than in earlier reported experiments. The inhibitory effect is still noticeable at 100 times lower concentration, but at that concentration the action can be noticed only in certain types of caries.

The latitude of action for I-ac can be determined, based upon LD_{50} or in this case MLD, which is estimated to about 50 mg. per kilogram body weight. Knowing the daily food consumption, the daily intake of I-ac is calculated to an average of 27 mg. per kilogram body weight in the 0.02 per cent I-ac group. The minimum daily I-ac intake with definite caries-inhibiting effect, therefore, will be 2.7 mg. per kilogram body weight. The minimum lethal dose is thus about 20 times greater than the minimum daily caries-inhibiting dose. For practical purposes, this margin is too narrow. It must be kept in mind, however, that the caries-inhibiting dose is not administered as a single dose, but distributed with the food intake throughout the day. Under these conditions there has been no evidence of toxic symptoms.

The experiment supports the assumption that the addition of glycolysis-inhibiting drugs to a caries-producing diet influences the pathogenic mechanism of the caries process. Although the I-ac does not seem completely suitable for this purpose, the experiment may serve as a basis for comparison with other caries-inhibiting compounds having similar action.

SUMMARY

In comparing the I-ac inhibitory effect in in vitro saliva-glucose-enamel tests with the in vivo effect on caries in rats, the in vitro tests show 10 times the inhibitory effect shown by the animal tests. The difference is modified depending upon which type of caries is used for the calculation. A distinct caries inhibition is obtained with an I-ac concentration of 0.002 per cent in the food.

The LD_{50} in peroral administration of I-ac in rats has been determined to be 116 ± 13 mg. per kilogram body weight.

The safety margin of I-ac is too narrow to permit its practical application as a caries prophylactic. The absence of toxic changes in experimental animals confirms the applicability of the principle of supplementing caries-producing diet with compounds having local action such as I-ac. For this purpose substances of lower acute toxicity must be found.

In the study of drugs inhibiting the glycolysis of dental caries, this experimental procedure may serve as a basis for comparison.

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TOXICITY TRIALS WITH CETYLAMINE

J. MALCOLM, M.D., F.R.S. (EDIN.), EILEEN MARY DEAKER, M.H.SC., AND MURIEL EMMA BELL, M.D.

From the Nutrition Research Department, Medical School, Otago, New Zealand

IT has been suggested by Walsh and Green[1] that solutions of high molecular weight, primary aliphatic amines in medicinal paraffin oil may be of value in prevention of caries. As no information was available concerning the possible toxicity of such compounds, it seemed desirable to conduct tests with experimental animals before proceeding to clinical trials.

EXPERIMENTAL PROCEDURE

Six weanling male white rats of Wistar strain averaging 80 grams in weight, with 6 similar controls were maintained on Coward's stock diet consisting of: yellow maize, ground finely, 65.0 parts; whole wheat, ground finely, 20.0 parts; caseinogen, 9.0 parts; dried brewer's yeast, 5.0 parts; sodium chloride, 0.5 part; calcium carbonate, 0.5 part; dried milk, 20.0 parts; iron citrate, 0.1 part; and potassium iodide, 0.1 part. In addition to this mixture, which was supplied to the rats ad libitum, about 4 Gm. cabbage per rat per week was given. Liver, which is usually included, was in this instance omitted in case it contained protective elements that might mask the possible toxicity of the cetylamine.

The experimental animals were given 4 drops of a 1 per cent paraffin solution of cetylamine daily by pipette, the daily dose being 0.0011 Gm. cetylamine. This would correspond initially to a dose for a 70-kilo man of 0.96 Gm. daily and, when the rats attained 300 gram weight, to a dose of 0.26 gm. daily.

A human being brushing on 1 to 2 c.c. of a 1 per cent solution once or twice daily would actually receive a maximum of 0.04 Gm. daily, if he swallowed the full amount, whereas most of the solution would probably be rejected from

TABLE I

WEIGHTS OF RATS AT END OF FIVE MONTH EXPERIMENT

	EXPERIMENTAL SERIES (GM.)	CONTROL SERIES (GM.)
	340	319
	357	291
	382	319
	272	291
	341	308
	305	381
Mean	332.8	318.2
Standard deviation	39.3	32.9
Standard error of mean	16.0	13.4
t = 0.70		
Difference is not significant.		

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the mouth. The dosage applied in the test on the rats was exaggerated for the purpose of magnifying any supposed toxic effects, especially with the possibility in mind of variation in species susceptibility.

For all of the rats, the experiment lasted for at least 5 months, after which period they were sacrificed 1 at a time at intervals for histologic examination. The final survivors had been maintained on the experimental dosage for 9 months.

RESULTS

The average growth curves are shown in Fig. 1. In Table I the individual weights in the experimental group are compared with the controls.

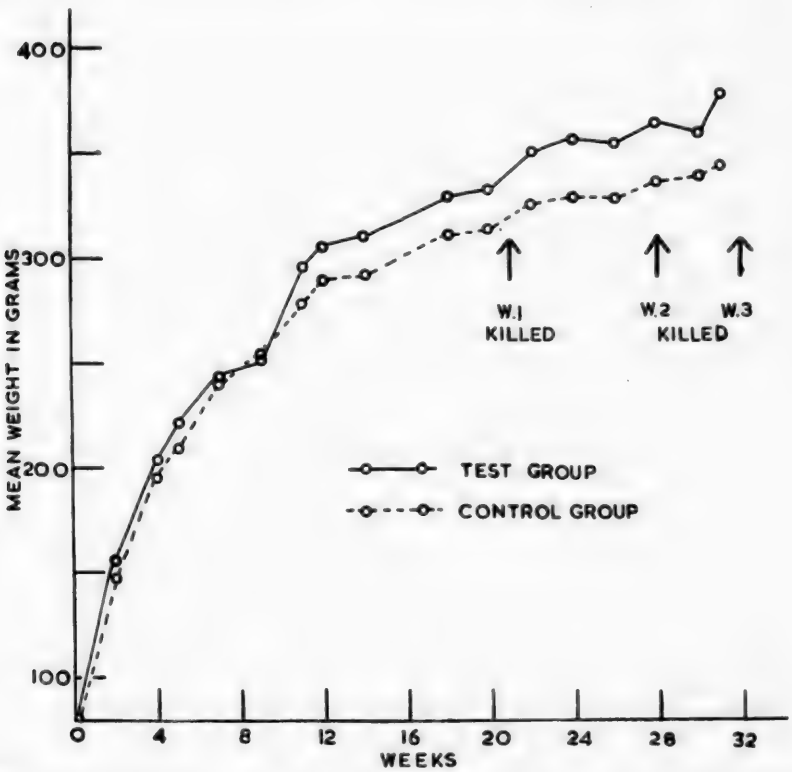

Fig. 1.—Growth curves of rats. The animals in the test group each received a daily dose of 0.0011 Gm. cetylamine in medicinal paraffin oil.

The average weight of the rats receiving cetylamine was slightly higher than that of the controls, but applying the "t" test of "Student," the difference was found not to be significant.

Histologic Examination.—Rats were killed at the following times from the beginning of the experiment: W.1, 21 weeks; W.2, 28 weeks; W.3, 32 weeks; then one experimental rat, W.4, and one control rat, W.5, 34 weeks.

The liver, spleen, pancreas, and testis of Rats W.1., W.2., W.3., and the thyroid and parathyroid in Rats W.1. and W.2. were examined and no evident abnormality found in any case. The only organ that seemed abormal was the kidney in each of the first 3 experimental rats. There were clusters of red blood corpuscles at a few points in the cortex and the tubules contained some stainable material. A comparison was then made between the kidney of an experimental rat (W.4.) and that of a control rat (W.5.) treated simultaneously in the same way in each case. No difference could be detected and we conclude that the cetylamine has no deleterious effect on the kidney or other organs when given in the dosage used in this experiment. The stainable material may be connected with the cloud observed in the albumin test referred to below.

Urinalysis.—Three experimental rats were kept in a Hopkins' metabolism cage for the purpose of collecting their urine. In order to avoid contamination from particles of food carried in their fur, the rats were given a fast period by day but allowed to feed at night. The urine collected by day was centrifuged and examined for red cells and casts, but none were found. The coagulation test for albumin gave a cloud, but as it has been demonstrated[2] that normal urine from mature male rats gives this test, the amount was compared with that obtained on testing 3 controls similarly kept in a metabolism cage. No difference was observed in the amount present, comparing the urine of controls and experimental animals.

DISCUSSION

Cetylamine is an aliphatic primary amine, $C_{16}H_{33}NH_2$, which, by virtue of its long hydrocarbon chain, possesses surface-active properties. It is, therefore, relevant to compare the observations recorded above with reports by other workers on both amines and surface-active materials. Probably the most extensive investigation was carried out by Walter and Carlson[3] who tested 69 surface-active agents of many different types. Although their method of intraperitoneal injection into mice may prevent direct application of their results to our problem, it is clear that this class of substance covers the whole range from quite innocuous to highly toxic. Nonionic emulsifying agents, such as polyoxyethylene monostearate, have been shown by Harris, Sherman, and Jetter[4] to be toxic to rats and hamsters when fed in quantities varying from 5 to 25 per cent of a nutritionally complete diet.

All these substances have complex chemical structures with several different polar groups to which any observed toxic effects might be ascribed. When we consider simpler amines, we find no reports of toxicity in the aliphatic series. The effect of ingestion of an aromatic amine was studied by Ewins and Laidlaw[5] who gave 0.5 Gm. of para-hydroxyphenylethylamine in a capsule to an 8-kilo dog. There were transient symptoms of stimulation of the sympathetic nervous system following a latent period of one-half an hour. The symptoms persisted for about 1 hour and then subsided. Para-hydroxyphenylacetic acid was recovered from the urine, and subsequent perfusion experiments indicated that the liver was especially responsible for the transformation, while heart muscle brought about destruction of the amine. Johnson[6] studied a number of amines

by injection into animals and found that, while para-phenylenediamine was markedly toxic, and pyridine and quinoline produced general anesthesia, the aliphatic diamies, cadaverine and putreseine, were nontoxic. Bell, Gregory, and Drummond[7] gave 2 mg. isoamylamine per rat per day for a period of 100 days without adverse effects on the growth rate, the dose being dissolved in arachis oil and fed directly into the mouths of the animals. The authors concluded that the tissues of the rat were capable of detoxicating the amine.

It appears that little or no toxic action is associated with primary aliphatic amines and, in view of the findings reported here, we consider that the small quantities of cetylamine which might be ingested in clinical trials would certainly have no harmful effects.

SUMMARY

Rats fed on a nutritionally complete diet showed no significant change in growth rate when dosed with cetylamine in medicinal paraffin oil for a period of nine months.

Histologic examination and urinalysis showed no difference between the control and experimental animals.

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THE PROTECTION OF THE WET ENAMEL SURFACE BY ADSORBED FILMS

R. W. GREEN AND J. P. WALSH

University of Otago Dental School, Dunedin, New Zealand

IT HAS already been shown[1] that mineral oil containing a small quantity of an aliphatic amine in solution can displace saliva from the surface of a wet tooth and form an oily film with the power of greatly inhibiting decalcification of the enamel surface by acid. In an attempt to throw more light on the nature of this effect, we now describe experiments made with a number of similar protective agents acting on intact tooth enamel and on powdered tricalcium phosphate.

EXPERIMENTAL METHODS

Since this study was regarded as a preliminary to a clinical investigation, the amines were used in their readily available commercial form without further purification. They contained in most instances about 90 per cent of the principal constituent together with 10 per cent of other amines from the same homologous series. Impurities with other polar groups were present in only very small amounts. To avoid confusion, we shall refer to the commercial materials by their proprietary names, and in Table I we reproduce information supplied by the manufacturer* concerning their composition.

It will be noticed that the number appearing in the proprietary name refers to the carbon chain length of the predominant molecular species.

Solutions of the amines in oil were made by dissolving the appropriate quantity in medicinal paraffin oil as described previously. Other substances used as solutions in medicinal paraffin were oleic acid, palmitic acid, and stearamide. Since high molecular weight amines are almost insoluble in water, to prepare aqueous solutions they were converted to their acetates by mixing with a slight excess of glacial acetic acid. The acetates were then dissolved in water and adjusted to pH 4.0 with more acetic acid.

In the earlier work, the decalcifying medium consisted of pooled human saliva brought to pH 4.0 by the addition of a concentrated lactate buffer. An experiment was made to discover whether the presence of saliva had any effect on the results. Using Method II of our previous paper,[1] a 1 per cent solution of Armeen 16D (cetylamine) in medicinal paraffin was tested against lactate buffer at pH 4.0 on several groups, each of 30 teeth, with the results shown in Table II.

It will be seen that the amine solution is distinctly more effective in the complete absence of saliva (Groups B and C) but that there is very little difference between Groups D and E. That is, it is immaterial whether buffered saliva or buffered water is used as decalcifying medium, provided the teeth themselves are wet with saliva before the oily solution is applied. The results

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*The Armour Laboratories, Chicago, Ill.

TABLE I

PERCENTAGE COMPOSITION OF COMMERCIAL ALIPHATIC AMINES*

PRIMARY AMINE	CARBON CHAIN LENGTH	PROPRIETARY NAME				
		ARMEEN 10D	ARMEEN 12D	ARMEEN 14D	ARMEEN 16D	ARMEEN 18D
Octylamine	8	3				
Decylamine	10	90	9			
Dodecylamine	12	7	90	4		
Tetradecylamine	14			90		
Hexadecylamine	16			4	90	6
Octadecylamine	18		1	2	6	93
Octadecenylamine	18				4	1

*Information supplied by The Armour Laboratories, Chicago, Ill.

of Groups B and C demonstrate that the oily solution is equally effective whether it is brushed on the teeth or merely shaken in the acid medium. The somewhat greater inhibition in Group C probably can be traced to the rather larger quantity of protective agent used. As a consequence of these observations, the use of acidified saliva was abandoned in favor of the more convenient pure aqueous buffer, the teeth being thoroughly wetted with saliva before beginning an experiment.

TABLE II

EFFECT OF DECALCIFYING MEDIUM AND INITIAL STATE OF TEETH ON ACTION OF ARMEEN 16D (CETYLAMINE) IN MEDICINAL PARAFFIN OIL

GROUP	INITIAL STATE OF TEETH	APPLICATION OF OIL	DECALCIFYING MEDIUM (PH 4.0)	MEAN* WEIGHT OF CALCIUM DISSOLVED IN 20 HOURS (MG.)
A	Wet with water	None	Water	7.69
B	Wet with water	Brushed	Water	2.42
C	Wet with water	Shaken in decalcifying medium	Water	1.89
D	Wet with saliva	Brushed	Water	3.72
E	Wet with saliva	Brushed	Saliva	4.05

*Each value represents the mean for 30 teeth. Standard error of each mean = 0.109 mg.

The experimental method used for evaluating the influence of any solution in reducing the susceptibility of teeth to decalcification was similar to Method I described in the earlier paper,[1] except that the teeth were polished with wet pumice after the wax had been removed by carbon tetrachloride. It was found that this more vigorous cleansing of the surface enhanced the protective effect of the oil film. Consequently, the results given below for cetylamine are appreciably higher than those previously reported. Unless otherwise stated, the oily solutions were applied by brushing on the saliva-wet tooth, while the aqueous solutions were added directly to the decalcifying medium.

Decalcification was brought about as before by buffer solutions of pH 4.0. It was found, however, that the same degree of protection was afforded by a given agent whether lactate or acetate buffers were used. Therefore, in the section dealing with aqueous amines, acetate buffers were employed, as being more compatible with the amine acetates. The buffers were prepared at 2M concentration and the appropriate amount was taken to produce a 0.2M solution in each experiment.

The effect of aqueous amines on dissolution of calcium phosphate was examined as follows. The correct quantities of stock buffer and amine acetate

solutions were mixed and diluted to 10 ml. in water, and then 50 mg. powdered calcium phosphate B.P. was introduced. The mixture was agitated at 37° C. for 30 minutes, centrifuged, filtered through a sintered glass crucible, and the calcium content of the filtrate was measured. The result was compared with a control experiment performed simultaneously in the absence of amine.

The method of Clark and Collip[2] for estimating calcium was found suitable only at low amine concentration. When the latter was much greater than 10^{-4}M, it appeared that amine was adsorbed on the calcium oxalate, resulting in the attachment of air bubbles to the precipitate, and preventing sedimentation in the centrifuge.

It became necessary, therefore, to remove the bulk of the amine before precipitating the calcium oxalate, and, after a number of trials, the following method, depending on the reaction between nitrous acid and primary amino groups, was adopted:

To 2 ml. calcium solution were added 1 ml. of 0.1M sodium nitrite solution and 1 ml. of 0.1M sulfuric acid. The solution was heated on the water bath for 10 minutes. An addition of 0.5 ml. M urea solution was then made to destroy the excess nitrous acid, and the mixture was warmed for a further 10 minutes. On cooling, a curdy precipitate appeared which probably consisted of the alcohol corresponding to the original amine, perhaps mixed with the sparingly soluble amine sulfate. The solution was filtered through a sintered glass crucible and 1 ml. saturated ammonium oxalate solution was added to the filtrate and washings. The pH was adjusted to 5.0 with a few drops of strong ammonia, and the precipitate of calcium oxalate was treated according to the method of Clark and Collip.

This procedure gave true results on amine solutions containing known amounts of calcium. However, the deaminated solution was unsuitable for colorimetric phosphate determination, as it appeared still to contain sufficient amine or by-product to interfere with that reaction. The several protective agents were therefore studied for their effect on calcium dissolution only.

EXPERIMENTAL RESULTS

Solutions of 1 per cent oleic or palmitic acid in paraffin were found to have no value in reducing acid decalcification of the teeth. Stearamide is only sparingly soluble in paraffin oil, and its solutions, too, were ineffective. Aqueous solutions of cetyltrimethylammonium bromide, a cationic surface-active agent, and of lissapol N, a non-ionic agent manufactured by condensing ethylene oxide with an alkylated cresol, had no action on decalcification. As cetyl alcohol had already been examined[1] and found inactive, attention was concentrated on a more complete study of the anemias.

It was found that, in addition to their protective action when applied in paraffin oil, the amines were equally effective when simply added to the decalcifying medium in the form of their water-soluble acetates. In Table III we show the data for both oily and aqueous amine solutions. The former consisted of solutions of 1 Gm. amine in 100 Gm. medicinal paraffin oil applied to the saliva-wet teeth, while the latter were added to the decalcifying buffer solution to a concentration of 5×10^{-4}M. The degree of protection is evaluated, as before,

as the percentage reduction in calcium dissolution from the protected side, as compared with the unprotected control side of the tooth. Each result represents the mean of measurements on 10 teeth.

TABLE III

DEGREE OF PROTECTION BY AMINES AT pH 4.0

AMINE	1 PER CENT SOLUTION IN MEDICINAL PARAFFIN		5×10^{-4}M IN DECALCIFYING BUFFER SOLUTION	
	MEAN PROTECTION (PER CENT)	STANDARD ERROR* OF MEAN	MEAN PROTECTION (PER CENT)	STANDARD ERROR* OF MEAN
Armeen 10D	1	3.5	2	5.1
Armeen 12D	12	2.3	11	4.4
Armeen 14D	86	0.9	76	6.5
Armeen 16D	60	2.1	37	3.2
Armeen 18D	34	2.9	41	3.2

*Nine degrees of freedom.

Since tetradecylamine appeared to afford the tooth surface greater protection than did any of its homologues, experiments were made to discover how effective it might be at lower concentrations. Table IV shows that the influence of the amine falls off rapidly as its solution in oil is diluted.

Teeth which had been dipped in aqueous amine solutions before placing in acid were protected only insofar as they carried with them some of the bulk solution of amine. Rinsing with water after the aqueous amine treatment completely removed any protective effect.

TABLE IV

EFFECT OF CONCENTRATION OF TETRADECYLAMINE IN PARAFFIN OIL

CONCENTRATION OF ARMEEN 14D (GM./100 GM. OIL)	MEAN PROTECTION AGAINST BUFFER SOLUTION AT pH 4.0 (PER CENT)	STANDARD ERROR* OF MEAN
0.1	− 3	5.0
0.3	+16	6.3
0.6	41	4.8
1.0	86	0.9

*Nine degrees of freedom.

Considering natural oils of the triglyceride type as solvents for the amine, we find that they all contain more or less free fatty acid, which can be expected to react with the amine forming a much less soluble oleate or palmitate. To overcome this difficulty, the acid value of a sample of olive oil was measured, and an amount of amine was then added to leave an excess of 1 Gm. amine per 100 Gm. oil after all the free fatty acid had been neutralized. This solution, when freshly prepared, was found to be just as effective as the solution in paraffin, showing that the action of the amine is independent of the nature of the oil in which it is dissolved. However, the solution in olive oil differed from that in paraffin in that it quickly lost its protective power and after several days was quite inactive. This can probably be attributed to a saponification reaction between the glyceride and the amine, resulting as before in the formation of an insoluble amine salt of the fatty acid. Such a reaction can be expected to be common to all fatty oils, so that medicinal paraffin appears to be the more suitable solvent.

In presenting the results of experiments with calcium phosphate powder, for the sake of uniformity, we again use the concept of degree of protection, that is, the reduction in calcium dissolution compared with a control test carried out simultaneously in the absence of amine. At the end of the 30-minute reaction period, it was found that the control solution contained about 8 mg. calcium in 10 ml. Fig. 1 shows how the degree of protection varied with the nature and initial concentration of the amine.

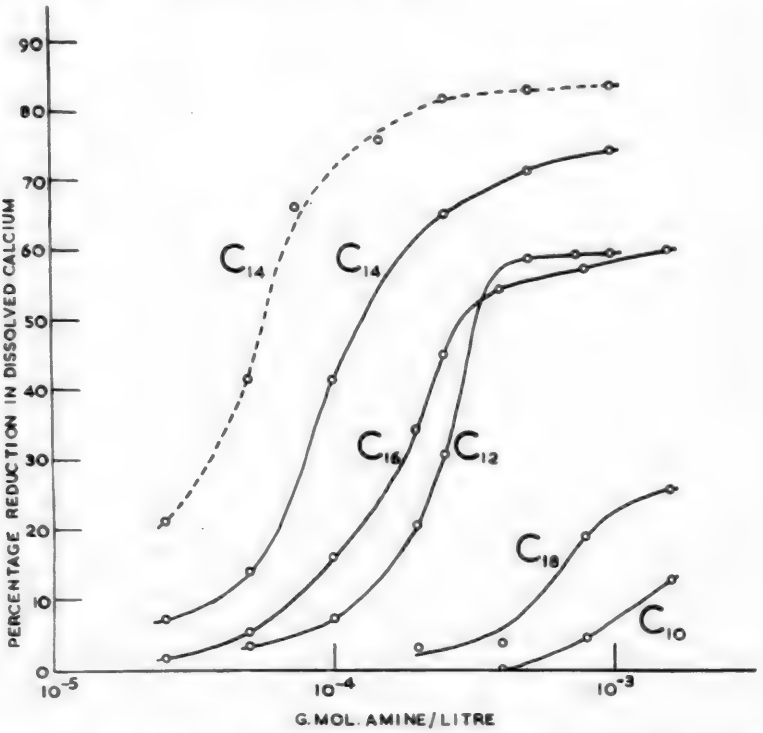

Fig. 1.—Effect of initial concentration and chain length of primary amines on the dissolution of calcium phosphate by an acetate buffer solution at pH 4.0.
Continuous curves = 50 mg. calcium phosphate in 10 ml. buffer solution.
Broken curve = 20 mg. calcium phosphate in 10 ml.

DISCUSSION

The experiments described above have shown that the action of acid on either intact extracted teeth or powdered calcium phosphate can be strongly inhibited by the presence of quite small concentrations of high molecular weight amines. This property was not exhibited by any of the other long-chain compounds studied, with the exception of oleic acid,[1] which possessed it to a very slight extent. Other acids, alcohols, amides, and the more complex detergents all seem to be quite inactive. The protection of enamel, therefore, differs widely from the inhibition of corrosion of metals, since the latter can be brought about by a wide range of polar groups.[3]

Although the effect appears to be restricted to a few amines, it is not due to their bulk properties as bases which can neutralize acid, since the amine acetates at pH 4.0 are just as active as the free amines in oil. Table II shows that the protective activity of the amine is closely dependent on the length of the carbon chain, passing through a sharp maximum for tetradecylamine, $C_{14}H_{29}NH_2$, and virtually vanishing when the chain is shortened to 10 carbon atoms. The amines with an odd number of carbon atoms in the molecule were not examined, so that it is possible that either the C_{13} or the C_{15} member of the series might be even more effective than tetradecylamine. From Table I it is also clear that some of the action of Armeen 12D must arise from the 9 per cent of tetradecylamine which it contains.

Qualitative observations of the sedimentation of calcium phosphate powder from aqueous amine solutions indicated that amine was being adsorbed on the particles of powder. The close correspondence between the maxima of the curves in Fig. 1 and the data of Table III supports the view that the protective action of amines on teeth is a consequence of their affinity for the calcium phosphate of the enamel. While there are many examples of "physical" adsorption of polar molecules on solids, and no further explanation may be needed than the postulation of Van der Waals forces between the polar group and the adsorbent, it is possible that the special position which the amines occupy in relation to calcium phosphate may be connected with the low solubility of their phosphates.

The effects reported here may be considered in the light of work by Bigelow, Pickett, and Zisman[4] and Shafrin and Zisman[5] who found that hydrophobic monomolecular layers could be deposited on platinum foil from solutions of amines and of many other long-chain organic compounds in water or nonpolar solvents. These authors, too, found that tetradecylamine occupied a unique place in the homologous series of the amines. It seems reasonable to suppose, therefore, that in our work the protective effect resides in a monomolecular layer of amine adsorbed on the enamel surface.

There are indications that a mobile equilibrium exists between the protective layer adsorbed on the tooth surface and the amine in the bulk of the solution, since, in the absence of oil, rinsing such a tooth in water destroys the effect. Consequently, the clinical use of aqueous amines appears less promising than that of solutions of amines in oil. The oil spreads in a relatively thick film over the monomolecular layer and should act as a reservoir for replenishing the adsorbed amine wherever it is required. At the same time, its viscous nature should provide a mechanical shield between the monomolecular layer and the decalcifying medium. The data of Tables II and III demonstrate that the protective effect will persist as long as any amine remains in the neighborhood of the tooth, since its affinity for the material of the enamel evidently causes it to be adsorbed on any exposed areas.

The curves of Fig. 1 emphasize the great importance of chain length. Differences among the several amines are accentuated here by the much greater surface area of the adsorbent. The calcium phosphate powder adsorbs considerable quantities of amine from solution, so that the final concentration of the latter may be much lower than it was initially. For each amine, under the conditions of our experiment, there was a critical concentration below which the

effect on calcium phosphate dissolution was only slight. The two curves for Armeen 14D, which differed only in the weight of calcium phosphate added, show that the critical value must be related, not only to the bulk solution, but also to the surface of the solid. Either of these factors may predominate. In experiments with teeth, the area is so small that the bulk concentration will be decisive.

The wide range of critical concentrations and of protective maxima in Fig. 1 cannot be fully explained in the present state of our knowledge. It is well known that the surface tension of very dilute solutions may take many hours to become constant, because of the slow diffusion of active molecules to the liquid-air interface; a similar phenomenon, at the solid-liquid interface, may have occurred at the lower concentrations used here. The equilibrium between adsorbed and dissolved amine, even when established, no doubt depends on packing of the different hydrocarbon chains, and is further disturbed by the continual dissolution of calcium phosphate and, presumably, the return of adsorbed amine to the solution. All these factors still await investigation.

SUMMARY

1. The investigation of the role of surface-active agents in inhibiting enamel decalcification has been extended to include 5 high molecular weight amines and other polar compounds.

2. The power to protect the intact enamel surface against acid was found only in the amines and was exerted from either aqueous or oily solution.

3. In the homologous series of primary aliphatic amines, the protective effect rises to a sharp maximum with tetradecylamine, $C_{14}H_{29}NH_2$. Under the conditions of our experiment, a 1 per cent solution of this amine in medicinal paraffin produced a degree of protection of 86 per cent. The protective effect was enhanced by thorough cleansing of the tooth surface before wetting with saliva.

4. Amines which have the ability to protect the intact enamel surface produce a parallel effect on powdered calcium phosphate by retarding its dissolution in acid to approximately the same extent. This demonstrates that amines protect the enamel by virtue of their affinity for its inorganic constituents.

5. The most suitable agent for a clinical investigation appears to be a solution of tetradecylamine in medicinal paraffin oil.

The authors wish to thank the members of the dental profession in New Zealand who supplied the sound teeth used in this work.

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THE PREVENTIVE ROLE OF FLUORINE AGAINST CARIES

THEMIS MAVROGORDATO, M.D., D.D.S.

Euangelismos Hospital, Athens, Greece

DURING the last decade extensive clinical and laboratory research all over the world and especially in the United States has established that fluorides exhibit a preventive effect on dental caries. Fluorides have been utilized in two different forms: either by the addition of sodium fluoride in a solution of 1 part per million in the nonfluorinated domestic water supply, or by the local application of a 2 per cent solution of sodium fluoride to the coronal surface of the teeth.

Two theories of the action of fluorine on tooth surfaces are prevalent today. The first, and more generally accepted, is chemical, i.e., that the fluorine of sodium fluoride replaces the less stable OH of the hydroxyapatite and forms calcium fluoride which resists the action of acid. The second theory is the one which applies to the local effect of fluorinated drinking water. It is based on the fact that the mouths of people living in areas using naturally fluorinated domestic water present an absence or reduction of lactobacilli which is believed to be due to the presence of fluorine.

OBSERVATIONS

Endemic mottled enamel was known to exist in the small Greek mining town of Lavrion, 45 miles southeast of Athens. Although the dental lesions had been considered as possibly due to fluorides in the domestic water supply, no one had verified this assumption. Some even attributed the lesions to lead and copper which exist in abundance in the mines of Lavrion.

I undertook, with the help of my assistants, to ascertain (1) the presence and the amount of fluorine in the domestic water of Lavrion which comes from a deep artesian well by the name of Bella Noria, (2) the extent and degree of the endemic mottled enamel in Lavrion, (3) the rate of dental caries among the Lavriotes, as compared with that of individuals in other Greek districts with a fluorine-free domestic water supply, and (4) the fluorine content of the domestic water supply of districts outside Lavrion, and the degree of dental caries among their inhabitants.

This study commenced in October, 1947. Our first object was to examine the domestic water supplies of Lavrion, and to verify the existence of fluorine and its percentage. With the cooperation of Dechaume of France, Deverell of England, and H. T. Dean of the United States, we were able to employ the Elvove zirconium and alizarin colorimetric method to determine the presence of fluorine and its percentage. A fraction over 3 parts per million were found.*

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*During his last visit to Athens in May, 1948, Dr. R. Sognnaes of Harvard was supplied by us with a sample of Lavrion water for examination. Mr. Rubens, of the Massachusetts State Laboratories, verified our finding and fixed the percentage at 3.2 parts per million.

EXAMINATION OF PATIENTS

During twelve long visits to Lavrion we examined the mouths of 1,200 people with mirror and explorer.

From general examination we gained the impression that dental caries, regardless of the age of those examined, was far less prevalent than among the people of other cities, especially of Athens with which we were so well acquainted. Among the 1,200 examined, 607 were school pupils and young factory workers between the ages of 6 and 18 years. For convenience, we decided to limit our study and comparison to this group of children between the ages of 6 and 18, instead of the 12 to 14 year group commonly used by our American colleagues.

The children were divided into two groups. The first, from 6 to 12 years, had a combination of deciduous and permanent teeth, and the second, from 12 to 18 years, had only permanent teeth. These categories were further classified into (1) those who were born and reared in Lavrion and also those who came to this town before the age of 1 year, all those whose permanent teeth were calcified in Lavrion (461); (2) those who had been born and had lived elsewhere and had established themselves permanently in Lavrion after the formation of their permanent teeth (146).

Varying degrees of mottled enamel were found to be present on all the permanent teeth of all those children born and reared in Lavrion and who had used the water of the artesian well (Bella Noria), and yet despite the superficial and occasional deep destruction of the enamel and the varying degrees of discoloration of the teeth, the explorer rarely entered to any depth in the dentin, finding, as a rule, a hard, resistant and nonyielding surface.

RESULTS

Only 4.81 per cent of the 291 children from 6 to 12 years old who were born and raised in Lavrion or who moved there at 1 year of age showed caries; this means that 95 out of 100 children were entirely free from caries. Among each 100 children examined, 44 teeth were decayed. Of these 44 carious teeth only 6 were deciduous. The 170 individuals from 12 to 18 years whose teeth calcified in Lavrion presented only 22.3 per cent caries (about 78 per cent were free from caries). There were 135 decayed teeth per hundred individuals examined in this group.

From the 146 children born elsewhere who came to Lavrion after the calcification of their teeth had been completed, the group of 118 from 6 to 12 years presented 14.6 per cent caries with 84 teeth decayed per 100 children. The 28 children between 12 and 18 years revealed 42.8 per cent caries, with 165 teeth decayed per 100 children.

Two points which impressed us most from the foregoing results, and which as far as we know have not been stressed in the literature that we have read, are as follows. (1) The deciduous teeth of children from 6 to 12 years of the first group (Lavrion born), although free from mottled enamel, presented a higher immunity to caries than the permanent teeth of Lavrion born children

of 12 to 17 years. Since the deciduous teeth of these children were generally free from fluorosis, we must conclude that the 3 parts per million of fluorine in the drinking water imbibed by the mother did not penetrate, as such, through the placenta to the embryo, but in a concentration not stronger than 1 to 1.5 parts per million (which we know does not produce fluorosis). In this way, without producing fluorosis there was enough fluorine incorporated in the tooth substance of those children to protect them from caries. (2) On the basis of the caries occurence in the 146 children not born in Lavrion in whom the formation of teeth was completed elsewhere and who later established themselves there, we came to the conclusion (contrary to some writers) that the coronal part of the Lavrion children's permanent teeth, having been bathed continually for years in a 3 parts per million solution of drinking water, retains part of the fluorine in its substance and becomes stronger and more resistant to acid attack.

Outside of Lavrion, for comparison, we examined 4,000 children of other districts, together with samples of their domestic water proved to be free from fluorine or containing only traces of it.

Our results were as follows.

Athens: 281 children from 6 to 12 years: 65 per cent caries and 266 carious teeth per 100 children; 344 children from 12 to 18 years: 70 per cent caries with 308 carious teeth per hundred children examined.

Island of Rhodos: 297 children from 6 to 12 years: 51 per cent caries and 86 carious teeth per hundred children; 382 children from 12 to 18 years: 64 per cent caries, 107 carious teeth per hundred children examined.

Northern Greece: 654 children from 6 to 12 years: 48 per cent caries, 126 carious teeth per 100 children; 448 children from 12 to 18 years: 43 per cent caries, 104 carious teeth per 100 children.

Archangelos Rhodos: 179 children from 6 to 12 years: 33 per cent caries, 54 carious teeth per 100 children; 89 children from 12 to 18 years: 42.8 per cent caries, 165 carious teeth per hundred children.

Katerini: 216 children from 6 to 12 years: 41 per cent caries; 168 children from 12 to 18 years with 59 per cent caries.

SUMMARY AND CONCLUSIONS

(1) The drinking water of Lavrion contains over 3 parts per million fluorine and produces mottled enamel of varying degrees on the permanent teeth of all children born and reared there.

(2) Despite fluorosis, the percentage of caries as compared with other Greek districts is very small.

(3) The nonmottled deciduous teeth of children born in Lavrion present a higher immunity to dental caries than the permanent teeth with fluorosis. This may be due to the smaller percentage of fluorine passing through the placenta to the embryo, and also to the local effect on the coronal surface of the deciduous teeth of the fluorinated drinking water after birth.

(4) Children who lived elsewhere so that the formation of their teeth was completed by the time they came and established themselves in Lavrion presented a lower incidence of caries due to the local action of fluorine on the coronal part of the teeth.

Teeth of Lavriotes as compared with those of people of other districts as, for example, Athens, Rhodos, and so on, present less caries regardless of being attacked by fluorosis or not.

In Northern Greece and other districts such as Archangelos Rhodos, Katerini, Crete, and so on, the incidence of caries is quite low, averaging 40 to 50 per cent. This we believe is due to other factors than fluorine, including diet with low consumption of sugar.

OXIDATION OF GLUCOSE DEGRADATION PRODUCTS IN THE PRESENCE OF SALIVA AND POSSIBLE RELATION TO CARIES IMMUNITY

J. C. CALANDRA AND ERNEST C. ADAMS, JR.

From the Chemistry Department, Dental School, Northwestern University, Chicago, Ill.

THE development of dental caries has been attributed to the formation of acids by the degradation of fermentable sugars in the mouth. Yet, in spite of huge intakes of sugar, some individuals have a natural immunity to dental caries. This immunity often appears independent of both hereditary and environmental factors. Attempts to explain this natural immunity have been based on the presence of some inhibitor of acid-producing organisms and on the absence of some coenzyme that is necessary for the acid production. Up to the present time such attempts have given inconclusive results. There is another possibility that appears more likely. The degradation of glucose is known to proceed to lactic acid over a series of intermediate compounds. If any of the intermediates or lactic acid itself could be oxidized rapidly to less acidic substances, the acid remaining in contact with the teeth would be reduced and a lower incidence of caries would be expected. Thus it is desirable to investigate the possibilities of the presence in saliva of enzyme systems capable of causing such oxidations.

An organism, *Micrococcus lactilyticus*, capable of causing the oxidation of lactate and only lactate has been isolated from the oral cavity. The products of the oxidation are propionate, acetate, carbon dioxide, and water.[1] Lewkowicz[2] was the first to discover this organism. Hall and Howitt[3] and Foubert and Douglas[4] further investigated the organism. Douglas[1] found the organism to occur in saliva in numbers ranging from 3×10^6 to 340×10^6 per milliliter. In some cases 50 per cent of the oral flora was made up of *Micrococcus lactilyticus*. This organism in the presence of lactate can cause a marked rise in pH when the medium is initially acidic because of the conversion of the strong lactic acid to the weaker propionic and acetic acids. Such oxidations then would quickly remove the conditions favorable for decalcification.

While there have been studies on oral organisms that are able to oxidize lactic acid, little is known about the enzymes that are produced by these organisms to cause this oxidation. Likewise, there has been little investigation of enzymes capable of catalyzing the oxidation of other glucose degradation products such as pyruvate, propionate, and acetate; however, such enzymes from tissue and other organisms have been investigated. Thunberg[5] showed that pyruvate in the presence of frog muscle causes the reduction of methylene blue. Others[6-10] have reported the oxidation of pyruvate by various animal tissues. The necessary enzyme for pyruvate also has been reported to be pres-

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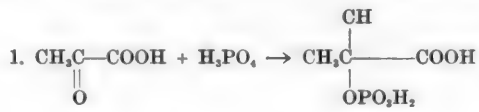
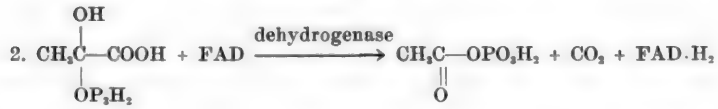
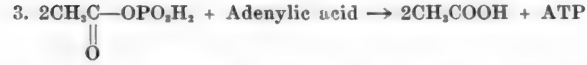
ent in certain bacteria.[11-14] Lipmann[13, 15, 16] obtained an acetone-dried preparation from *Lactobacillus delbrueckii* which oxidized pyruvate to acetate and carbon dioxide. Lipmann[17] reported that the oxidation of pyruvate required inorganic phosphate, thiamine phosphate, flavin adenine dinucleotide, magnesium ions, and the dehydrogenase. He represented the reaction as:







Such an oxidation would still leave an acid, but it would be much weaker and in turn could be oxidized to carbon dioxide and water. Pincus[18] demonstrated the presence of a pyruvic dehydrogenase in saliva and suggested that a lactic dehydrogenase may also be present. As to the nature of the pyruvate dehydrogenase, not much is known. Barron and Singer[19] suggested that a sulfhydryl group was essential in its prosthetic group as the oxidation of pyruvate was inhibited by SH reagents.

The aims of this study were to investigate the possibility of the presence in saliva of enzymes capable of causing the oxidation of various glucose degradation products and to express the activities of these enzymes on a quantitative basis.

EXPERIMENTAL

The study was carried out using the Warburg respirometer. The technic was that described by Umbreit.[20] The experimental procedure was as follows.

One milliliter of phosphate buffer (0.05M, pH 7.2) and 1 ml. of pooled saliva (collected by paraffin stimulation) were placed in the main vessel. One milliliter of a phosphate buffer solution of the alkali metal salt of the desired acid (0.1M) was placed in the side arm. Two tenths milliliter of 20 per cent potassium hydroxide was placed in the center well and a piece of fluted filter paper inserted to provide a greater surface for absorption of carbon dioxide. The ground-glass joint and the side arm stopper were greased lightly with anhydrous lanolin and the flasks were placed on the manometers and immersed in the water bath. The flasks were shaken for ten minutes to reach temperature equilibrium (bath temperature was 37.6° C.). Then the flasks and stoppers were frozen in by gently turning them back and forth. The contents of the side arms were emptied into the main vessels by tipping the manometers, and the initial readings were taken. Readings were taken every ten minutes after that for the next hour and every half hour for three and a half hours. Simul-

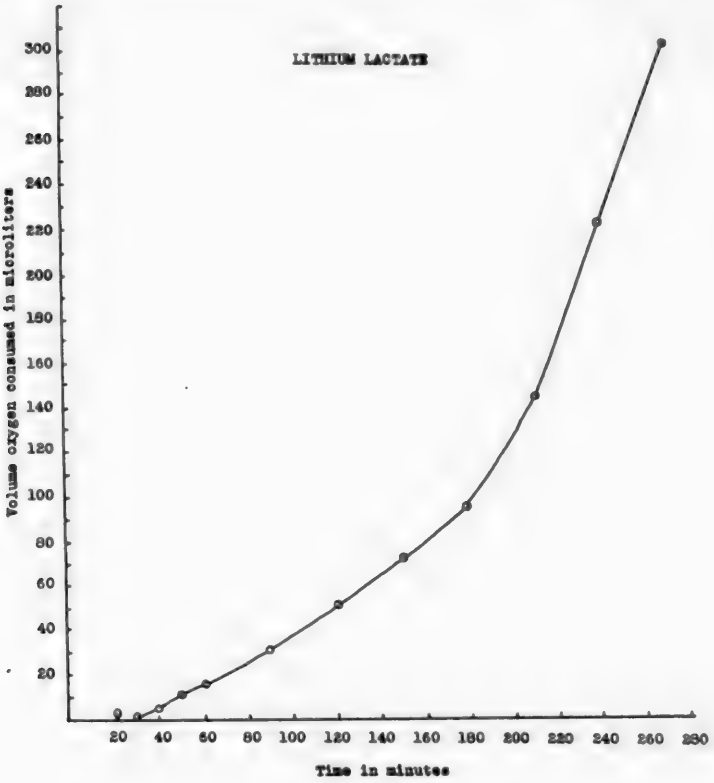

LITHIUM LACTATE
Volume oxygen consumed in microliters
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
20 40 60 80 100 120 140 160 180 200 220 240 260 280
Time in minutes

Fig. 1.

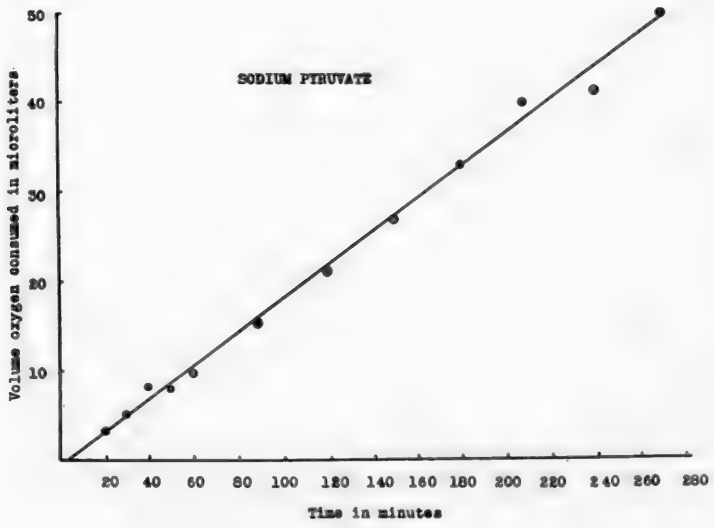

SODIUM PYRUVATE
Volume oxygen consumed in microliters
50
40
30
20
10
20 40 60 80 100 120 140 160 180 200 220 240 260 280
Time in minutes

Fig. 2.

taneously, controls containing everything except the substrate were run. The pressure changes were converted to volume change by means of a previously calculated graph. The difference between the total oxygen uptake and the

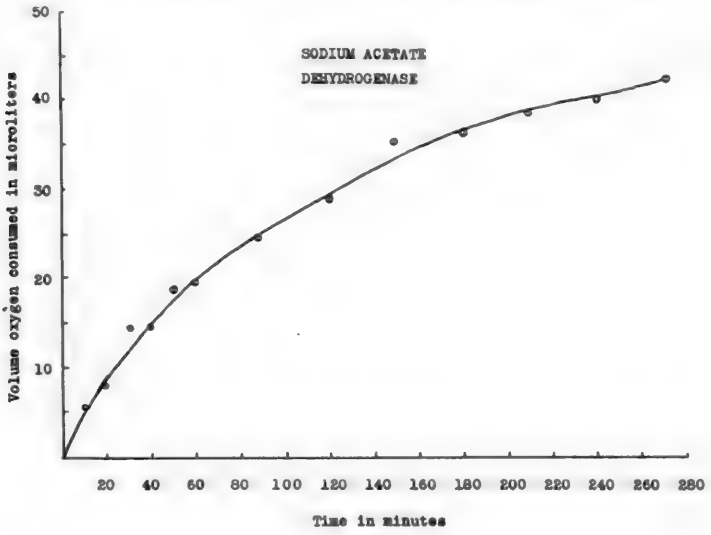


Fig. 3.

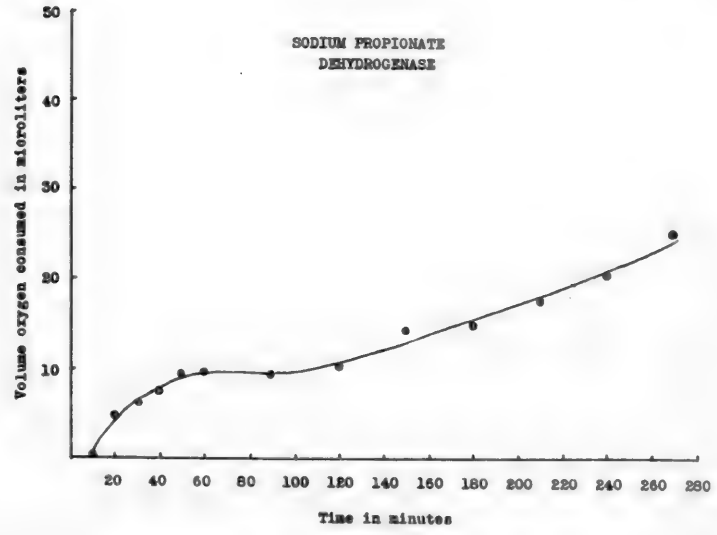


Fig. 4.

uptake control gave the oxygen uptake of the substrate itself. The oxygen uptake of each substrate in microliters was plotted against the time in minutes. These curves are shown in Figs. 1 to 4. The slopes of the curves were deter-

mined in microliters of oxygen uptake per hour. By dividing by 22.4 (microliters occupied by 1 μM of gas), the microliters of oxygen uptake per hour were converted to micromoles of oxygen utilized per hour. Since the exact course of the oxidations are unknown, unit enzymatic activity was defined as that activity per milliliter of saliva that will cause the uptake of 1 μM of oxygen per hour. These activities are given in Table I.

TABLE I

SUBSTRATE	ACTIVITY*
Pyruvate	0.5
Acetate	0.78
Lactate	6.9
Propionate	0.23

*Uptake in micromoles of oxygen per hour per milliliter of saliva.

DISCUSSION OF RESULTS

The results show that there is a slight oxidation of pyruvate and acetate and an appreciable oxidation of lactate. There was very little oxidation of propionate and no detectible oxidation of citrate, succinate, malate, or acetoacetate. The rapid oxidation of lactate is the most important. Such an oxidation would rapidly raise the pH of the medium if initially acid. The course of the lactate, pyruvate, and acetate oxidations is probably:

$$\underset{\substack{\text{OH}\\ \text{Lactic acid}}}{\text{CH}_3\text{—CH—COOH}} \xrightarrow{-\text{H}_2} \underset{\substack{\text{O}\\ \text{Pyruvic acid}}}{\text{CH}_3\text{—C—COOH}} \xrightarrow{-\text{CO}_2} \text{CH}_3\text{—CHO} \xrightarrow{\frac{1}{2}\,\text{O}_2}$$

$$\underset{\text{Acetic acid}}{\text{CH}_3\text{COOH}} \xrightarrow{2\,\text{O}_2} 2\text{CO}_2 + 2\text{H}_2\text{O}$$

The oxidation of pyruvate and acetate probably aids in removing the products of the lactate oxidation and thus allows a faster oxidation, although less lactic acid may be formed because of the oxidation of the intermediate pyruvic acid. Either interpretation of the oxidation of pyruvate will give the same result. From the results obtained here it appears that the presence of enzyme systems capable of causing a rapid oxidation of lactic acid is the most promising factor for the explanation of natural caries immunity. The source of these enzyme systems is undoubtedly the bacterial flora of the oral cavity. Further investigations are now under way to attempt to show a significant difference between the rates of oxidation of lactate, pyruvate, and acetate in the presence of caries-active saliva and caries-immune saliva.

SUMMARY

1. A rapid oxidation of lactate and slight oxidations of pyruvate, acetate, and propionate in the presence of saliva have been shown and measured by means of the Warburg respirometer.

2. The possible relation of these oxidations to natural caries immunity has been discussed.

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SOME PHYSICOCHEMICAL FACTORS IN RELATION TO THE CAUSATION OF INTERPROXIMAL CARIES

R. B. NEVIN AND J. P. WALSH

University of Otago Dental School, Dunedin, New Zealand

IN 1946 a proposed investigation into biophysical and physicochemical factors which may affect dental caries was approved by the Medical Research Council of New Zealand. Earlier papers[1-4] have dealt with various aspects of this project. This paper is concerned with another problem as outlined in the original report.

As Fosdick[5] has stated, "It is quite obvious that the only determining factors as to whether one will or will not have dental caries are the rate of acid formation and the rate of acid neutralization." He and his co-workers have been chiefly concerned with acid production and in discussing neutralizing factors; he briefly mentions "physical features, diet, oral hygiene and dentistry."

It has long been recognized that tooth form and the shape of contacts between teeth may influence the onset of dental caries, but little scientific work has been done. In recent years, surveys by Barr,[6] Walsh and Smart[2] for the deciduous dentition and by Shourie, Heim, Leung, Simmons, and Marshall-Day[7] and Barr[8] for the permanent dentition have shown that the incidence of caries on the mesial and distal surfaces of individual teeth may vary considerably. For example, there is a much greater incidence of caries on the distal surfaces of the first deciduous molar and the first permanent premolars than on the mesial surfaces of these teeth. The main observable variable for such a limited region is the physical shape of the interproximal contact area.

It has been suggested previously[1] that the rate of diffusion of the soluble products of bacterial activity from these "stagnant" areas of interproximal contact may be of importance in the inception of dental caries. Black's successful principle of "extension for prevention" and the often observed difference in susceptibility to proximal caries on the two sides of a mouth, in one side of which a tooth has been lost and the remaining teeth have separated a little, may also be explained in physicochemical terms.

The present investigation is concerned with the extent to which physicochemical factors, such as the shape and size of the interproximal space and the buffer capacity of the surrounding medium, will affect the rate of diffusion of acid from this space. This was done by measuring the changes in hydrogen-ion concentration of an acidified solution contained in the space between two parallel contiguous rods of Pyrex glass, when immersed in solutions of known composition. The shape of the "contact area" was varied in a controlled manner.

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Preliminary experiments indicated the necessity for using glass rods of low solubility. Variable results were recorded when attempting to utilize a softer type of glass than Pyrex, apparently because of solution of the glass. This effect, though minute in quantity, was quite enough to alter the composition of the small amounts of liquid required to fill the space. Previous experiments using pairs of human teeth with an electrode at the contact area were similarily unsuccessful, as the dissolution of enamel caused a rapid rise in pH of solutions below pH 5.0.

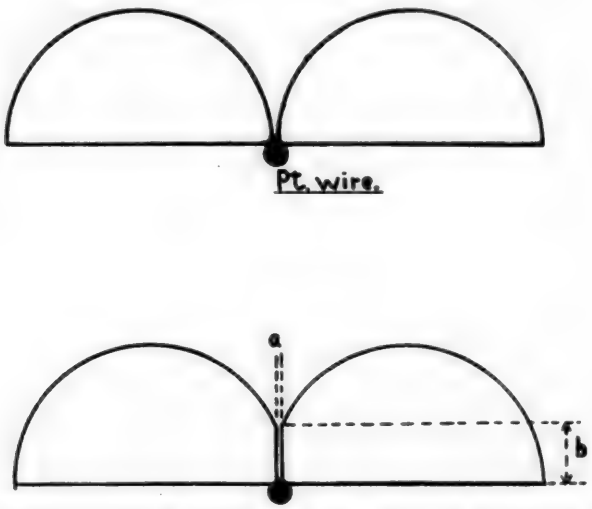

Fig. 1.—Cross section of glass rods; *a*, width of space, *b*, depth of space.

APPARATUS

Rods of Pyrex glass, 1.0 cm. diameter and 7.5 cm. long, were ground to accurate semicircular cross section and then mounted as parallel pairs with accurately measured gaps between them (Fig. 1). Some pairs were left as complete semicircles. Others had carefully measured flats ground and lapped along the adjacent parallel edges. This operation was carried out with pairs of rods luted together along the diametrical faces of the semicircles so that errors in angulation of the small flats were the same for each rod of the pair, thus ensuring perfect parallelism of the small flats when the rods were set up with the large flat faces coplanar, as in Fig. 1. The gap depth (b) was made 0 mm. (i.e. complete semicircles), 1.0 mm., 1.75 mm., and 2.25 mm., the gap width (a) for all these being made 0.14 mm. A duplicate of the first type (b = 0 mm.) was also prepared, having a gap width of 0.07 mm.

A piece of cleaned platinum wire 0.5 mm. in diameter was placed along the middle of the length of each gap, so as to make a tight seal against the sharp edges of the glass for 0.5 cm. of the length from one end of the wire, and sealed into place with a neutral wax The free end of the wire was bent outward

sharply and connected to an insulated lead. The sealing of the rest of the length of the gap on either end of the wire was completed by thin slips of glass, using very small amounts of wax to lute these to the coplanar flat faces of the semicircular rods. The insulated lead, the exposed platinum wire, and the back of these glass slips were then bonded strongly together with more wax melted evenly over the glass. Small crosspieces joining the end faces of the rods resulted in a space with sealed ends, contained between two slightly spaced curvatures and of length very much greater than the width. This construction ensured that diffusion of ions between the space and the surrounding medium could take place only at right angles to the length. The rate of change of pH of acid solutions placed in the space was measured by a Cambridge pH meter reading to within 0.01 pH. The platinum wire formed one electrode and a calomel half-cell connected through saturated potassium chloride solution formed the other, the liquid in the gap and the surrounding liquid both being saturated with quinhydrone. To prevent errors in readings caused by the liquid junction potential between the two solutions, and by changes in this potential due to ionic concentrations varying as diffusion proceeded, all the solutions used were saturated with potassium chloride.

TESTING OF APPARATUS

The dependability of the assembled apparatus was determined by comparing measurements made with it of the pH of a standard buffer against values determined by an external platinum electrode. After thoroughly cleaning the gap by copious washing with distilled water, using fine flattened platinum wire and thin filter paper as mechanical aids, very dilute hydrochloric acid was introduced, and the pH measurements compared in the same way. Lack of agreement for the acid measurements indicated that traces of buffer were held in the gap. This was due either to insufficient washing or to diffusion into some very fine space resulting from an inadequate sealing procedure during assembly of the apparatus. If further thorough and careful cleaning failed to rectify the error, then the apparatus was reconstructed and rechecked.

STANDARDIZATION OF EXPERIMENTAL PROCEDURE

To obtain a series of readings, a solution of potassium chloride acidified to the required pH by hydrochloric acid was placed in the gap, and the apparatus immersed in a beaker containing the "surround" solution. An immediate measurement was taken to check freedom from contamination, and then the subsequent changes in pH were read off at suitable intervals.

Graphing the results so obtained gave a curve showing the rate of change of pH, or the rate at which hydrogen ions are lost from the deepest part of the space between the rods. At the end of each experiment, the pH of the "surround" solution was checked for constancy. Even with an unbuffered solution, the minute amount of acid in the space produced a change of not more than 0.1 pH in 100 c.c. of solution.

For each particular set of conditions investigated, it was necessary to ensure that the results would be comparable. The most obvious variable in repetition of measurements would be the amount of liquid introduced into the gap, especially in the case of the completely semicircular rods. To control this by weighing was possible, but experimentally difficult. Visual estimation was therefore attempted.

A quantity of hydrochloric acid solution of pH 1.8 was introduced into the gap to produce a free surface which was 1.5 times the width separating the semicircular rods (Fig. 1, a = gap width = 0.14 mm.) as measured by a microscope with vernier eyepiece. The visual appearance as seen through a strong magnifying glass was noted also.

The apparatus was immersed in an acetate buffer and an experimental determination of rate of change of pH made. Two further experiments using visual estimation only were carried out. Fig. 2 shows the results obtained, and also the results of measurements made for amounts of liquid sufficient to give a free surface width (c) in Fig. 2, of 2.5 and 5.0 times the gap width, corresponding roughly to liquid depths (d) of two and three times that of the first amount.

For all subsequent determinations the liquid surface width was set at approximately twice the gap width. The small variation shown in Fig. 2 for the widely varying amounts of liquid in the gap demonstrated that the average of three sets of readings for each condition investigated was a valid figure.

Where the gap width was 0.07 mm., the liquid surface width was visually adjusted to approximately 0.2 mm., in order to give as nearly as possible the same depth of liquid to the platinum electrode in both cases.

EXPERIMENTAL RESULTS

The effects of the following factors, separately and in combination, were studied. Determinations for the first four factors were carried out on the completely semicircular gap formation.

1. Initial pH of the acid in the gap.
2. pH of the external solution (unbuffered).
3. Buffering capacity of the external solution.
4. Gap width.
5. Depth of gap (using the parallel-side type of gap).

Fig. 3 shows the changes in the rate of diffusion brought about by altering the initial pH of the acid from 1.6 to 0.6, the pH of the external potassium chloride solution being 4.9 in both cases. Fig. 4 shows the results when the acid was of constant pH 1.6 and the external pH was varied from 4.8 to 7.0 by the addition of traces of caustic potash to the potassium chloride solution.

The effect of using buffering agents in the external solution (Fig. 5) was very pronounced. Acetate buffer (sodium acetate and hydrochloric acid) of varying molarity was used. These results were so definite that the experiments were repeated, for gap widths of both 0.07 and 0.14 mm. using a phosphate buffer (KH_2PO_4 + KOH) of pH 7.0, which has its optimum buffering value round about this pH. The acid in the space was adjusted to pH 3.5. These

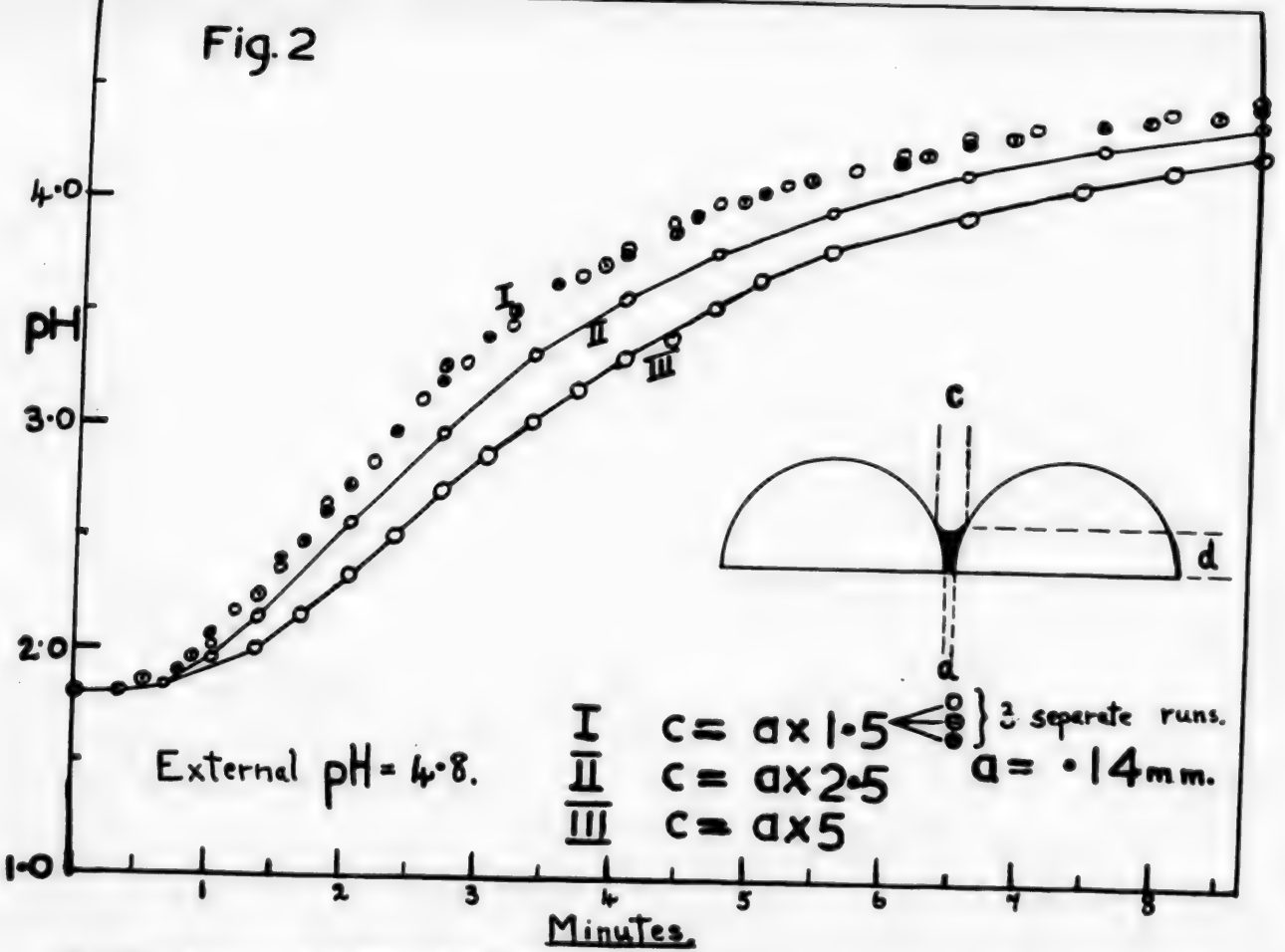

Fig. 2.—Effect of variation in amount of liquid introduced into gap. *c*, Width of free surface of liquid; *d*, depth of liquid.

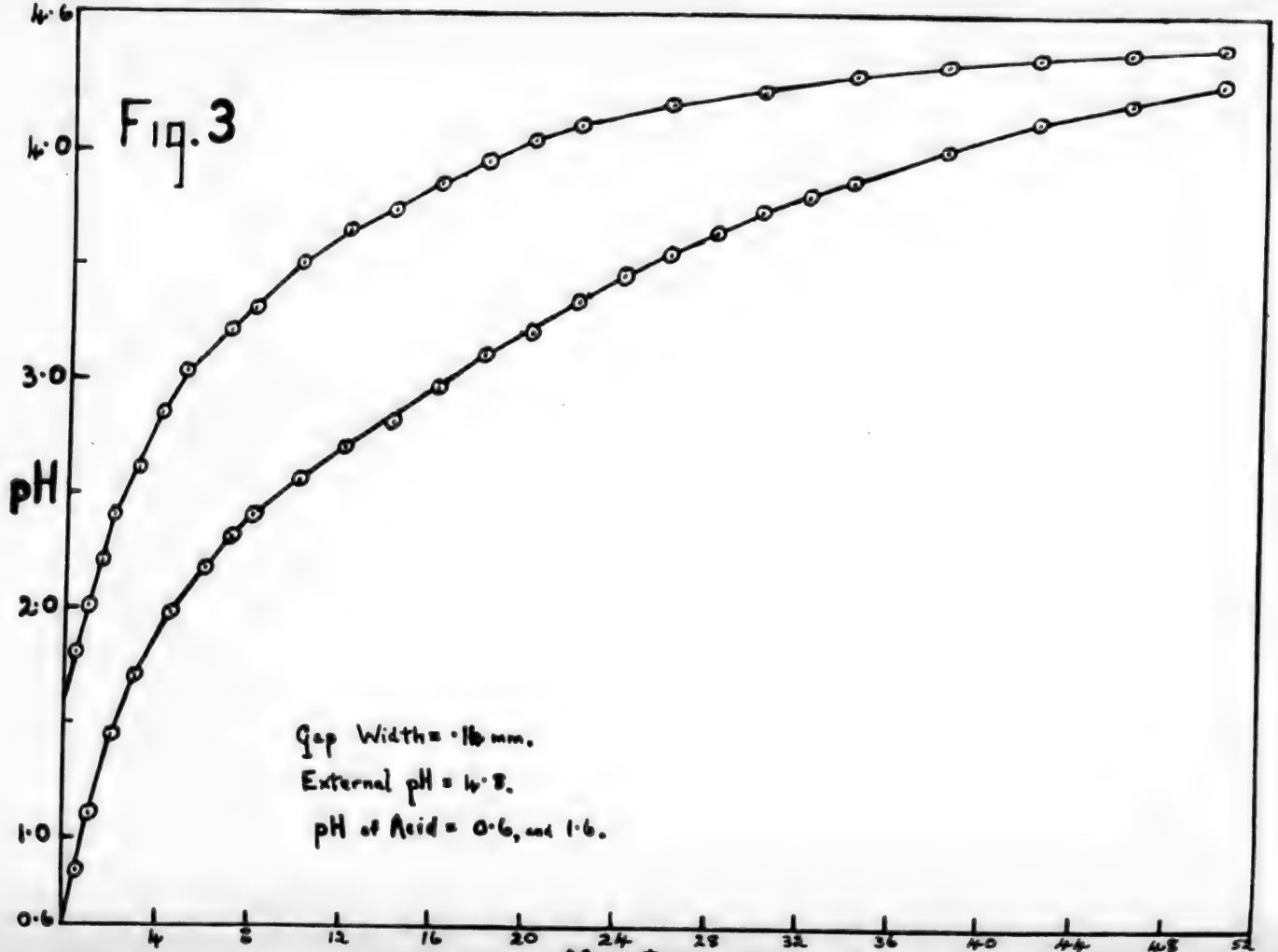

Fig. 3.—Effect of variation in initial pH of liquid in the gap.

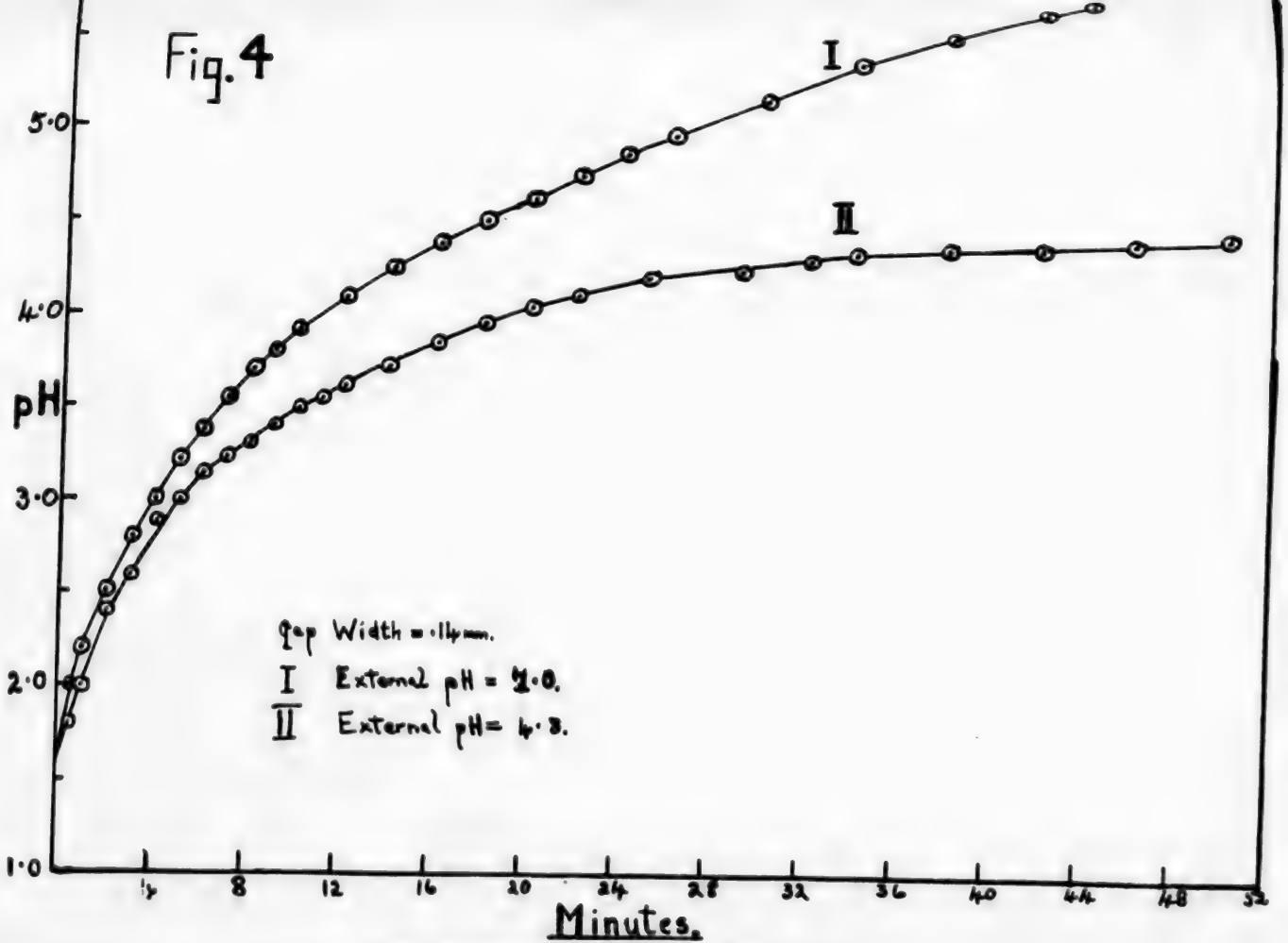

Fig. 4.—Effect of variation in pH of external solution.

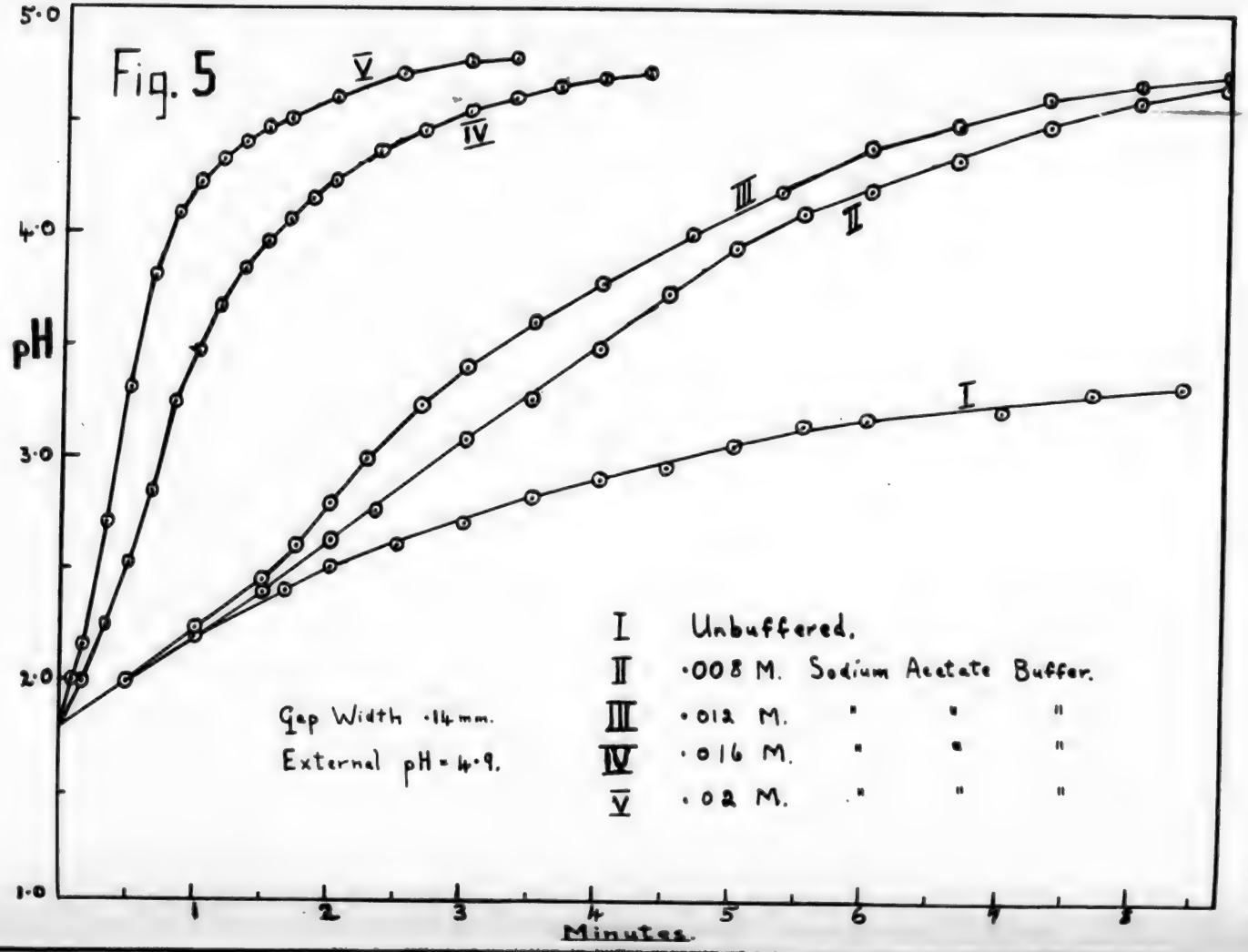
Fig. 5
pH
5·0
4·0
3·0
2·0
1·0
1
2
3
4
5
6
7
8
Minutes.
I
II
III
IV
V
I Unbuffered.
II ·008 M. Sodium Acetate Buffer.
III ·012 M. " " "
IV ·016 M. " " "
V ·02 M. " " "
Gap Width ·14 mm.
External pH = 4·9.

values were chosen in order to have some correspondence with the normal pH range of saliva, and the pH range that has been recorded on tooth surfaces during acid fermentation. The phosphate buffer was varied in buffering capacity as measured by the titrated volume of 0.1N lactic acid required to change the pH of 5.0 ml. of the buffer solution from 7.0 to 6.0. Buffer dilutions requiring 0.1 ml., 0.3 ml., 0.6 ml., and 0.9 ml. of lactic acid respectively were used, corresponding to the range of values that has been recorded for saliva (Dreizen, Mann, Cline, and Spies[9]). These strengths of buffer solution will from here on be referred to in terms of their buffer capacity as measured in this manner; e.g. a 0.1 titer buffer is one requiring 0.1 ml. of the lactic acid for the above-mentioned change in pH.

From the dilution factors required to reduce the standard phosphate buffer to these buffer capacities, the molar concentrations were deduced. The results are shown in Fig. 6 for a gap width of 0.07 mm. The effect of variation in gap width is exemplified in Fig. 7, where results for the two widths of gap are compared.

The final series of determinations was carried out on different depths of the gap, a constant gap width of 0.14 mm. being used. Fig. 8 is produced from the averages of the values recorded. In Fig. 9 a comparison is made between the effects produced by two different buffer capacities for two different depths of the gap.

DISCUSSION

Effects of Hydrogen-Ion Concentrations and Buffer Agents.—Comparison of Figs. 3, 4, and 5 shows that while the initial concentration of acid and the relative hydrogen-ion concentrations in the gap and outside it both have an effect on the rate of diffusion, this is quite small for the period of time during which the pH rises by 2 units, i.e., a decrease of the hydrogen-ion concentration by a factor of 100. Over this same pH range, however, the presence of quite low concentrations of buffer agents is sufficient to increase the rate of diffusion very considerably (Fig. 5), although it can be seen that at the lowest concentration of the buffer, the initial slope of the curve is identical with that recorded for absence of buffer. This last point will be referred to later.

Variation of Buffering Capacity.—The results recorded for the series where buffer capacities are of values corresponding to the range for human saliva (Figs. 6 and 7) seem to throw some light on the variable degree of correlation attained in attempts that have been made to link up the buffer capacity of the saliva with the degree of caries susceptibility. Increasing the buffer capacity beyond the value recorded for human saliva does not materially affect the rate of diffusion. Again, comparison of the rates of diffusion obtained with variation in buffer concentration and in mechanical spacing (Fig. 7) reveals that although a ninefold increase in buffer capacity increases diffusion rate approximately three times, a slight increase in the width of the space of only 0.07 mm. achieves the same result. Hence the effects of any variations in buffer capacity can be largely obscured by very slight, practically undetectable variations in the physical conditions. The same relative increase in

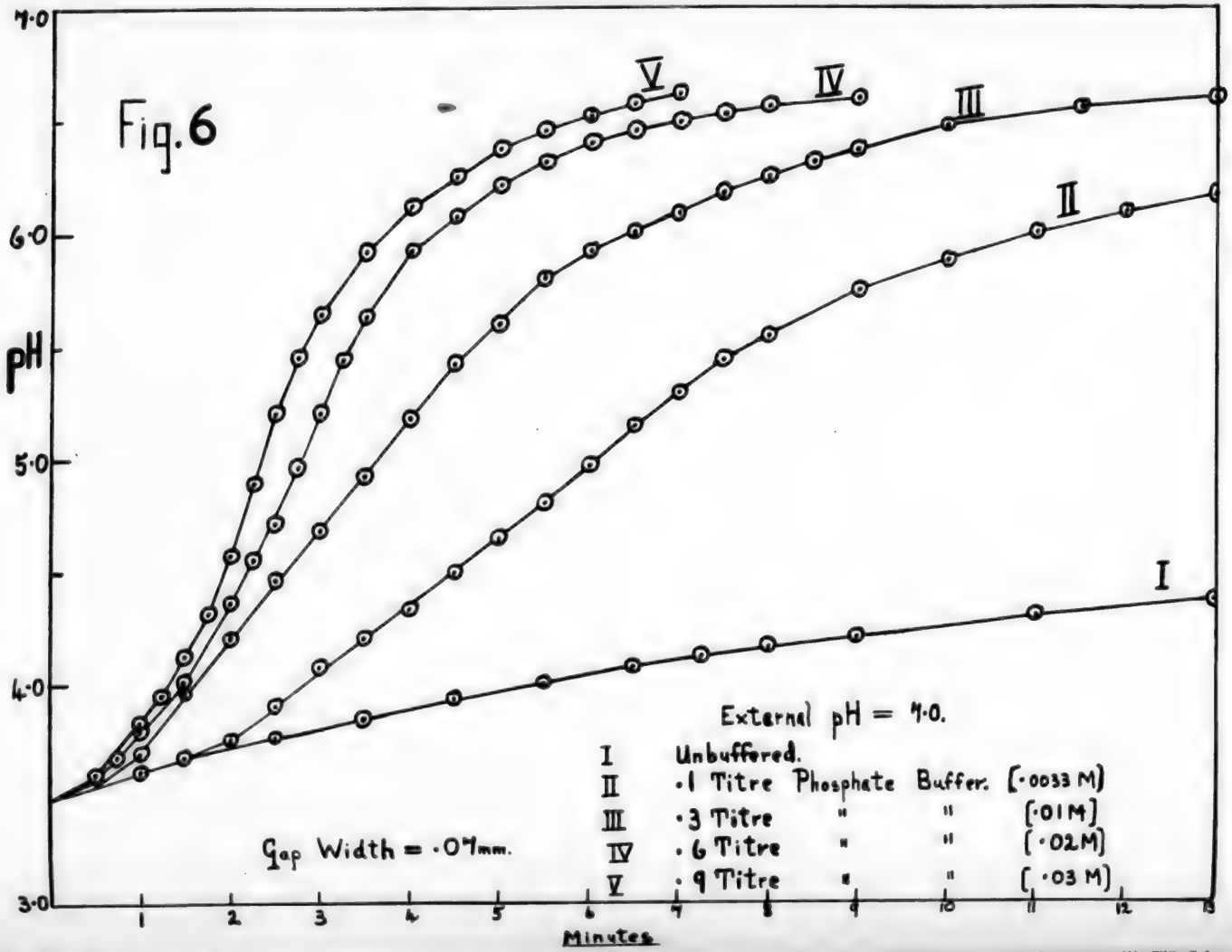

Fig. 6.—Effect of variation in buffer capacity of external solution using a different buffer and keeping the width of the gap constant at 0·07 mm.

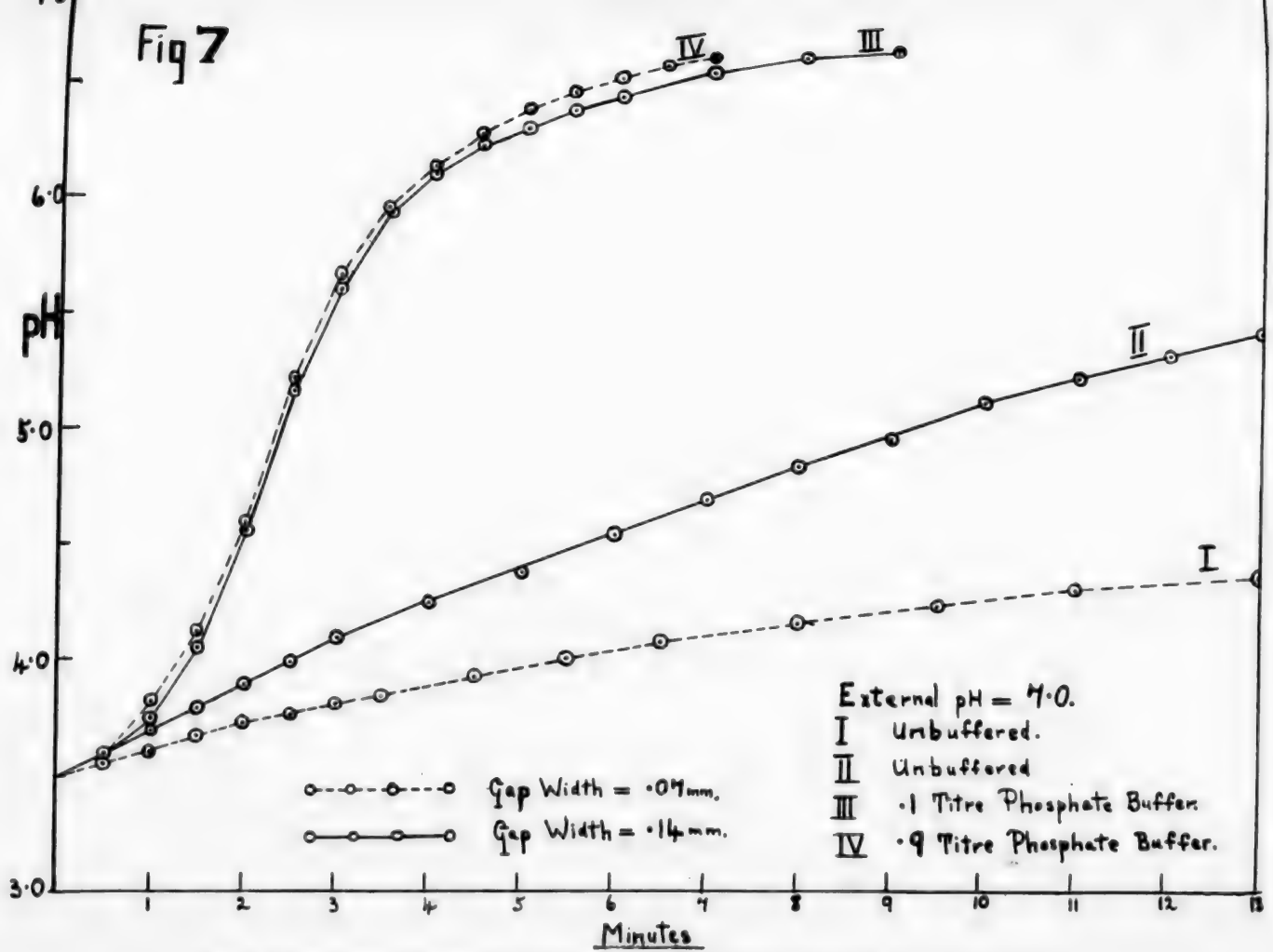

Fig. 7.—Comparison of effects of altering width of gap and buffer capacity of external solution.

Fig. 8
pH
Minutes
a = 1·4 mm.
External pH = 4·0 (·3 Titre phosphate Buffer.)
I b = 2·25 mm.
II b = 1·75 mm.
III b = 1·00 mm.
IV b = 0·0 mm. (semicircular pits).
I
II
III
IV
a
b

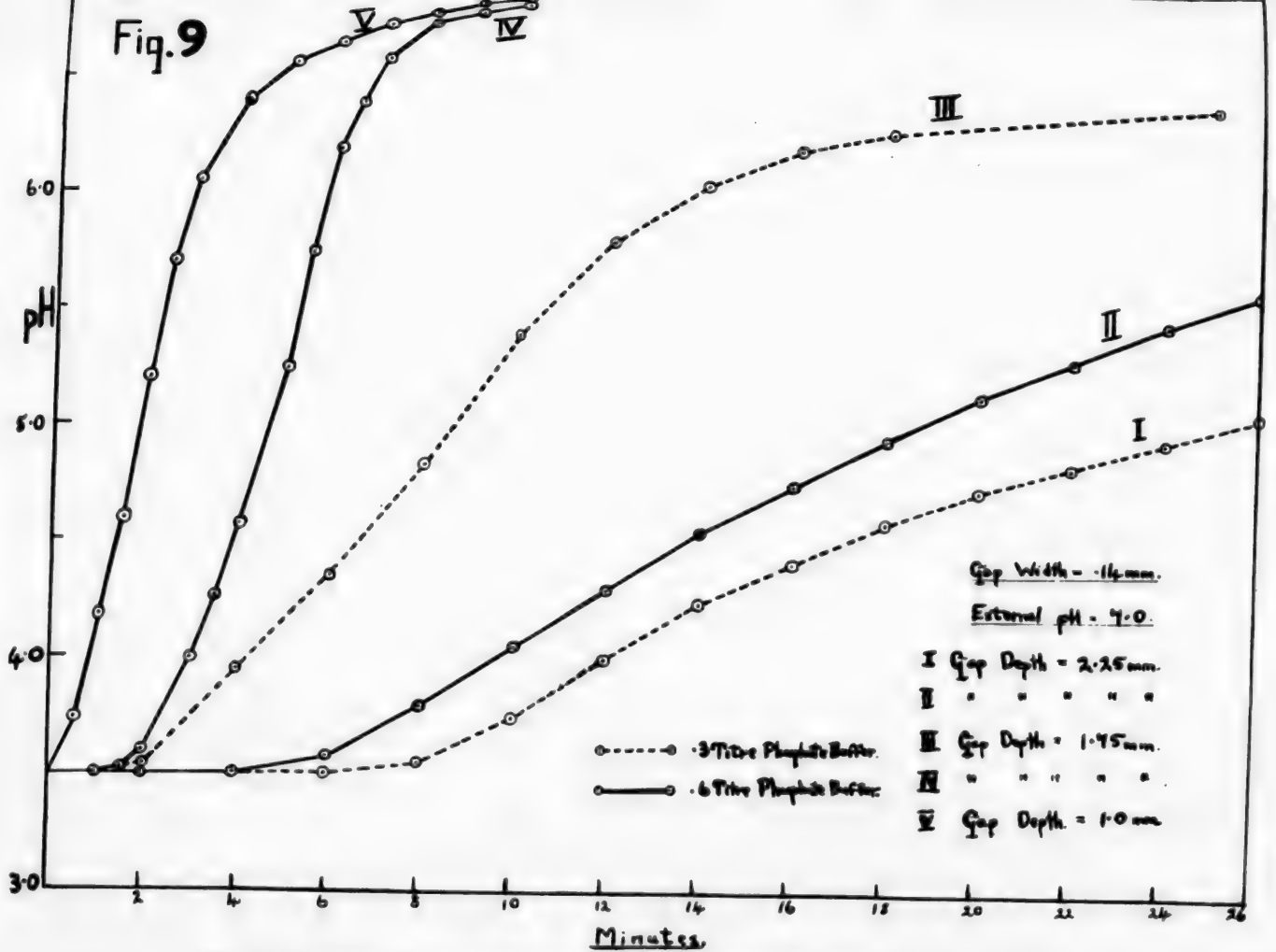

Fig. 9.—Comparison of effects of altering buffer capacity of external solution and depth of gap.

diffusion rate obtains also for the unbuffered condition, showing that the effect is due to physical conditions alone. The profound effect of the depth of the space on diffusion rate is seen in Fig. 8. The transition from "spherical contact" to a "line or area contact" is accompanied by a great decrease in the rate of diffusion, even in the presence of an external buffering agent. Experimental difficulties associated with a very narrow deep space set the lower limit for width at 0.14 mm., but if it is taken as an approximation from the results in Fig. 7 that halving this width will decrease the rate of diffusion by another three times, then it is not difficult to form a concept of the degree of "stasis" that must exist at the middle of the contact area between two molar teeth with flattened interproximal surfaces, even when there is nó other impediment to free diffusion. Fig. 9 shows that this effect is relatively more pronounced with higher external buffer capacity. Doubling the buffer capacity is twice as effective for a gap depth of 1.0 mm. and 1.6 times as effective for a gap depth of 1.75 mm., when compared to a gap depth of 2.25 mm.

Correlation of Results With Clinical Knowledge.—From all these results, it can be seen clearly that the shapes and spacing of the approximating surfaces of the teeth are by far the most potent factors influencing the rate of removal of acid from the narrow space between them, and that a small change in these dimensions (Figs. 7 and 8) will override the effect of other variable factors, such as the initial concentration of the acid (Fig. 3) and the total volume of acid present (Fig. 2). This strongly suggests that the physical shape of the stagnant interproximal area will be of greater importance than the actual rate of acid formation in determining whether or not the hydrogen-ion concentration in the space will reach and stay at a sufficiently low value for long enough to damage the surface of the enamel.

In the case of a small portion of the dental arch, such as the first deciduous molar or first permanent premolar regions, the other factors involved will be reasonably constant, and it would seem that only on the basis of the foregoing concept is it possible to explain the considerable differences in caries susceptibility between the mesial and distal surfaces of these teeth. As indicated earlier, Barr[6] reported a maximum difference in susceptibility for the D/E contact as against the C/D contact, as a ratio of five to one (at 6 years of age), the ratio decreasing of course with increase in the age group; while for the same areas, Walsh and Smart[2] reported three to four times less susceptibility for the lower C/D space, and two to three times less for the upper C/D space, than for the corresponding D/E spaces.

In the case of the first permanent premolar teeth, this effect is especially marked in the lower teeth. Barr[8] reported a factor of 3, and Shourie, Hein, Leung, Simmons, and Marshall-Day[7] a factor of 2½ for the greater susceptibility of the distal surfaces of these teeth. This difference assumes significance when the results in Fig. 8 are viewed in terms of "spherical contact" and "line or area contact."

Mechanism of the Effect of Buffers.—A question of importance that arises from this work concerns the mode of operation of the buffering agents.

Besic[10] has demonstrated that substances in solution apparently diffuse with difficulty into narrow spaces, but it is possible that it is not absolutely essential for buffer agents to penetrate right into the deepest parts of such spaces in order to materially affect the rate of loss of acid from the space. The removal of the hydrogen ions from the less narrow parts of the space would tend to maintain the diffusion "gradient" at its original value, and would be important when the buffer agent could enter such a space only with great difficulty.

The results shown in Fig. 4 demonstrate that changing the pH of the plane at which the acid initially meets the external solution does not materially affect the first steep rise in pH. But buffers not only prevent a change in pH at this plane, they also greatly increase the slope of the diffusion rate (Fig. 6), and so would seem to penetrate a considerable distance into the space. This is also indicated by the sudden change from the initial slope, which at first is similar to the unbuffered condition. The really critical area of the space appears to be where the walls approach parallelism. This is borne out by the diminished effectiveness of buffers for a long narrow space, as illustrated in Figs. 8 and 9.

The relative lengths of time to reach the same pH of 5.1 at the electrode area in spaces with depths of 1.0 mm., 1.75 mm., and 2.25 mm. can be expressed as a ratio of approximately 1:2:5.5 for an external buffer capacity of 0.3 titer (Fig. 8). Doubling this buffer capacity alters the ratio to approximately 1:2.5:10.5 (Fig. 9). But even with this effect, a gap depth of 1.75 mm., equivalent to a contact area between teeth of 3.5 mm. length, shows a curve that is concave upward for most of the length. Only with a gap depth of 2.25 mm. is the curve concave downward, similar to the cases where diffusion takes place into an unbuffered "surround." Hence it seems likely that buffer ions in solution can diffuse quite rapidly through and into narrow spaces even when these are relatively deep. If it is desired to evaluate the rates of diffusion for a higher starting pH of the acid (say 4.0), and to obtain the times taken to reach the critical pH of approximately 5.2, below which decalcification of enamel will occur, then this can be done by reference to Fig. 3 (unbuffered diffusion). Fig. 8 shows that variation in factors such as spacing affects unbuffered and buffered conditions equally, and so the assumptions made in the transfer from the lower to the higer pH are valid.

SUMMARY

1. The conditions affecting the rate of loss of acid from narrow spaces between contiguous curved and flattened surfaces have been investigated.

2. The experimental method was designed to simulate some of the purely physicochemical factors obtaining in the dental arch.

3. Where the shape of the space and the acid concentration were constant, the effect of external buffer capacity was of importance, though tending to reach a limiting value.

4. The physical factor of spacing was of much greater importance than the buffering capacity of the external medium. An alteration in width of 0.07 mm. equalled the effect produced by a ninefold change in buffer capacity.

5. Increasing the depth of a parallel-sided space had a similarly very pronounced effect.

6. Variations in the shape of interproximal areas would seem to adequately explain differences in caries susceptibility for different interproximal surfaces of the same tooth, and may be fundamental to all variations in susceptibility between different parts of the dental arch.

7. The foregoing findings are consistent with the theory that acid decalcification of the enamel surface is the initial lesion of interproximal caries. Above the pH level at which acid decalcification begins (and for a constant rate of acid production), these results indicate that the resulting rate of fall in pH in interproximal spaces is dependent on (a) the degree of separation of the teeth and (b) the shape and width of the contact or capillarity areas, and to a much lesser extent, the buffering capacity of the saliva. Once the critical pH is reached, hydrogen ions will also be removed by their interaction with the inorganic constituents of the tooth enamel.

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OBSERVATIONS ON SALIVARY LACTOBACILLUS COUNTS FOR A PERIOD BEFORE AND AFTER TOPICAL APPLICATIONS OF TWO PER CENT SODIUM FLUORIDE

PAUL C. KITCHIN, HAMILTON B. G. ROBINSON, DOROTHY PERMAR, AND ANDREW H. IMHOFF

College of Dentistry, Ohio State University, and Dental Division, City Department of Health, Columbus, Ohio

DURING the past quarter of a century there have been numerous demonstrations[2, 4, 9, 11, 12] of a close correlation between the consistent presence of relatively large numbers of lactobacilli in the saliva and active dental caries, and conversely, a close correlation between consistently few or no salivary lactobacilli and low caries activity. Since it has been reported that topically applied fluorides tend to decrease the incidence of dental caries,[13, 14, 15] the question has arisen whether or not that effect is reflected in the salivary lactobacillus counts.

Dean, Jay, Arnold, McClure, and Elvove[7] reported an inverse relationship between the lactobacillus counts and natural fluorine content of communal water supplies in a study of Galesburg, Ill., where there is a relatively high natural fluoride content of the drinking water (1.8 ppm), and Quincy, with practically no fluorides in the domestic water supply. Similar correlations of high fluorine-low salivary lactobacillus populations were reported by Dean, Jay, Arnold, and Elvove[5] and by Jay and Arnold.[10]

Conversely, Boyd and Cheyne[1] reported a complete lack of correlation between fluorides in the domestic water supply and salivary lactobacillus estimations. It is to be noted, however, that their study of 308 children, whose average age was 15 years 9 months, included a significant percentage of subjects who had an unknown fluoride history for all but the last two to five years. There was no division of this questionable group into individuals with mottled and nonmottled enamel, the authors merely stating that some had mottled enamel. Those with nonmottled enamel may or may not have had sufficient fluoride at the critical time of tooth development to affect caries susceptibility. Boyd and Cheyne did not find a lessened number of high lactobacillus counts among the children with mottled enamel.

Finn and Ast[8] investigated the salivary lactobacillus counts in Newburgh, N. Y., where 1 ppm of fluoride has been added artificially to the domestic water supply, and in Kingston, N. Y., where the water is practically fluoride-free. At the end of two years they found increasingly more low lactobacillus counts among children from the city with artificially fluoridized water than had been found at the start of the water fluoride period.

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A 2 per cent solution of sodium fluoride topically applied to the teeth has been reported to reduce the incidence of dental caries in groups of preadolescent and adolescent children a maximum of 40 per cent.[13, 14, 15] Knutson[13] has stated, without presenting data, that he found no difference in the salivary lactobacillus counts between groups of treated and of untreated individuals.

The purpose of this paper is to present data collected on (1) salivary lactobacillus counts of children before topical sodium fluoride treatments, and for a period of time thereafter, and (2) salivary lactobacillus counts on a nontreated, but otherwise similarly observed, group.

METHODS

Two groups of children were studied. Group A consisted of twenty-eight children, aged 7 to 9 years at the start, who were living in an orphanage. Group B consisted of forty-nine children, 14 to 16 years at the start, who were attending school at the Ohio State School for the Deaf.

All of the children were given clinical examinations, and bite-wing radiographs were taken. Three salivary samples were collected at least one week apart and lactobacillus counts were made. Groups A and B were each divided into two subgroups, one subgroup in each to be experimental and the other to serve as control. So far as was possible the treated and control children were paired so that the members of each pair were similar as to sex, age, caries experience, lactobacillus counts, and previous experience with fluorides in the drinking water.

Each member of the control groups was then given an oral prophylaxis. Each member of the experimental groups was given an oral prophylaxis and a topical application of 2 per cent sodium fluoride, followed by three additional applications of sodium fluoride at about one-week intervals, according to the method described by Knutson and Armstrong.[14]

The three initial pretreatment salivary lactobacillus counts were followed by a number of post-treatment counts taken at intervals of two weeks to two or three months. In the case of Group A, the counts covered a period of 22 months. The counts on Group B covered a period of 11 months.

Because of a misunderstanding, six members of the control subgroup among the orphanage children (Group A) received sodium fluoride applications in the manner described at the end of a one-year observation period.

All salivary lactobacillus counts were made by the method described by Permar and Kitchin.[16]

DATA

Table I is a compilation of the salivary lactobacillus counts of 14 orphanage children, aged 7 to 9 years at the time of the first count. An oral prophylaxis and the first application of sodium fluoride were given each child on the day following collection of the specimen for count number 3 (count number 4 in the case of Subjects 13A and 21A). Subsequent sodium fluoride applications occurred on the day following collection of samples 4, 5, and 6,

TABLE I

SALIVARY LACTOBACILLUS COUNTS, COVERING A PERIOD OF 22 MONTHS, ON ORPHANAGE CHILDREN WHO RECEIVED TOPICAL APPLICATIONS OF 2 PER CENT NaF AT TIME MARKED * (EXPERIMENTAL)

SUBJECT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	SUMMARY
2A	0	0	0*	0*	0*	0*	0	0	0	0	0	0	+	±	0				No change
3A	±	+	+*	+*	++*	+++*	+	±	±	±									No change
6A	0	0	0*	0*	0*	++*	0	0	±	+++	++++	+++	++++	++++	++++	++++	++++		Increase
8A	+++	+	+*	++*	++++*	+++*	+++	+++	+	++	++	++++	++++	++++	++++	++++	++		No change
11A	0	±	0*	0*	±*	0*	±	±	±	0	0	±	0	0	0	0	0		No change
13A	0	0	0	0*	0*	0*	0*	0	0	0	0	0	0	0					No change
14A	+++	+++	++++*	++++*	+++*	+++*	++++	+	±	++++	++++	++		++++	++++	++++	++++	++	No change
15A	++++	++++	++++*	++++*	++*	++++*	++++	++++	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No change
17A	0	0	0*	0*	0*	+*	+	+++	++	++++	+++	+	++++	++++	++++	++++	++++		Increase
19A	0	0	0*	0*	0*	0*	0	0	0	0	0	0	0	0	0	++	++++		Increase (†)
21A	±	0	0	0*	0*	0*	0*	±	0	0	0	0	0	+++	++++	++++	++++	++++	Increase
25A	+	+	+++*	+++*	+++*	+++*	+++	++	++++	++++	++++	++++	++++	++++	++++	++++	++++		No change
27A	0	0	±*	0*	±*	0*	0	0	0	+	++	+	+++	++	++++	+	±	+	Intermittent
28A	++++	+	++*	++*	++*	±*	+	+	++++	++++	++++	+++	++++	++++	++++	++++	++++		No change

0 = No colonies on plate.
± = 100 to 1,000 lactobacilli per cubic centimeter saliva.
+ = 1,000 to 5,000 lactobacilli per cubic centimeter saliva.
++ = 5,000 to 10,000 lactobacilli per cubic centimeter saliva.
+++ = 10,000 to 50,000 lactobacilli per cubic centimeter saliva.
++++ = Over 50,000 lactobacilli per cubic centimeter saliva.
*Sodium fluoride treatment given day following sample collection.

TABLE II

SALIVARY LACTOBACILLUS COUNTS, COVERING A PERIOD OF 22 MONTHS, ON ORPHANAGE CHILDREN WHO RECEIVED ONLY A DENTAL EXAMINATION AND AN ORAL PROPHYLAXIS AT TIME MARKED † (CONTROL)

SUBJECT	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	SUMMARY
1A	0	+	+	+†	±	±	±	±	+++	+	++++	++++	++++	++++					Increase
4A	0	0	±†	±	0	±	±	0	0	0	0	±**	±	±	+	0	+		No change
5A	±	0	0†	0	0	0	0	0	0	0	+++	0**	0	0	0	0	0	0	No change
7A	+	+	+†	0	0	0	0	0	0	++	+	++++	±	0	0	+	0		No change
9A	0	0	0†	0	0	±	±	0	0	0	+	+**	±	±	±	++	++		No change
10A	±	+	±†	+++	+	+	+++	+++	±	+	+**	+++	+++	++	++++	+	++		Increase
12A	+	±	+†	0	+	++	+++	++	+	+++	+	+	+++	++++	++++	++++	++++	++++	Increase
16A	0	0	0†	0	±	±	+	±	+++	+++	+	++++	++++	++++	++++	++++			Increase
18A	0	0	±†	0	0	0	0	0	0	0	±**	+	++	++++	++				Increase
20A	0	0	0	0†	0	0	0	0	0	0	0**	0	+++	+	+				No change
22A	0	0	0†	0	0	0	0	0	0	0		0	0	0	0	0			No change
23A	+	0	0†	0	0	0	0	+	+++	+++	++++	++++	+++	++++	++++	++++	++++		Increase
24A	±	±	0†	0	±	0	0	0	±	0	0	0	0	++++	+	0	0		No change
26A	0	0	0†	0	0	0	0	0	0	0	0	0	±	±	++	±	±		No change

**Members of this control group accidentally treated with NaF at a later date.
†Oral prophylaxis given following collection of this specimen.

indicated by an asterisk (*) in Table I. These specimens were collected one week apart. Subsequent counts, 7 through 18, were made at somewhat irregular intervals of two to three months.

Table II contains the record of salivary lactobacillus counts of 14 children used as controls. One oral prophylaxis, without sodium fluoride application, was given following the collection of the specimen for the third lactobacillus count, or as indicated by the symbol † in Table II. Control children 4A, 5A, 9A, 10A, 18A, and 20A who accidentally received sodium fluoride applications at the end of one year's observation, as indicated by two asterisks (**), must, after these applications, be considered as belonging to the experimental group.

Table III contains the record of salivary lactobacillus counts of 25 children from the School for the Deaf, aged 14 to 16 at the time of the first count. An oral prophylaxis and a topical application of sodium fluoride were given each child after the third saliva specimen was collected. Three additional sodium fluoride applications were administered within the following three weeks. Salivary lactobacillus counts were made from specimens collected at the dates indicated for a period of nine months following the last sodium fluoride treatment.

Table IV is a compilation of the salivary lactobacillus counts of the 24 control children at the School for the Deaf. An oral prophylaxis, without fluoride application, was administered to each subject following collection of saliva specimen number three.

TABLE III

SALIVARY LACTOBACILLUS COUNTS, COVERING A PERIOD OF 11 MONTHS, ON CHILDREN FROM THE SCHOOL FOR THE DEAF WHO RECEIVED TOPICAL APPLICATIONS OF 2 PER CENT NaF FOLLOWING THE THIRD LACTOBACILLUS COUNT (EXPERIMENTAL)

SUBJECT	11/13 1	11/17 2	12/13 3	1/12 4	1/21 5	2/3 6	2/17 7	4/14 8	5/19 9	9/29 10	SUMMARY
25	++++	++++	++++	++++	++++	++++	++++	+++	++++	++++	No change
26	+++	++++	++++	++++	++++	++++	+++	++++	+++	++++	No change
27	++++	++	++	+++	±	++	0	±	+++	++	Intermittent
28	++++	++++	++++	++++	++++	++++	-	++++	++++	-	No change
29	+++	0	0	-	±	0	0	0	0	0	No change
30	++++	++++	++++	++++	++++	++++	-	++++	++++	++++	No change
31	++++	+++	+++	++++	++++	++++	0	0	0	++++	Intermittent
32	+++	±	++	++++	++++	++++	+++	±	++++	++++	No change
33	++++	++++	++++	++++	-	++++	++++	++++	++++	++++	No change
34	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No change
35	++++	++++	++++	++++	++++	++++	++++	+++	++++	++++	No change
36	+++	++++	++++	+++	+++	+++	0	-	±	+++	Intermittent
37	++++	++++	++++	±	±	+	+	+++	+++	++	Intermittent
38	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No change
39	++++	++++	++++	+	++++	++++	++++	++	-	++++	No change
40	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No change
41	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No change
42	+++	0	0	0	0	0	0	0	0	0	No change
43	++++	+++	+	-	++	++++	++++	±	±	+	Decrease (†)
44	++++	++++	++++	++++	++++	++++	++++	0	0	0	Decrease
45	++++	++++	++++	++++	++++	+++	++++	++++	++++	++++	No change
46	++++	++++	++++	++++	+++	++++	++++	++	+++	++++	No change
47	+++	+++	+	++	+++	++++	++	++	+	++++	No change
48	+++	++++	++++	++++	++++	++++	++++	++++	++++	++	No change
49	+++	++	0	±	+	0	0	±	-	-	No change

TABLE IV

SALIVARY LACTOBACILLUS COUNTS, COVERING A PERIOD OF 11 MONTHS, ON CHILDREN FROM THE SCHOOL FOR THE DEAF WHO RECEIVED ONLY A DENTAL EXAMINATION AND ORAL PROPHYLAXIS FOLLOWING THE THIRD LACTOBACILLUS COUNT (CONTROL)

SUBJECT	11/13 1	11/17 2	12/13 3	1/12 4	1/21 5	2/3 6	2/17 7	4/14 8	5/19 9	9/29 10	SUMMARY
1	++++	++++	+++	-	+++	++++	++++	++++	++	++++	No change
2	+++	++++	±	+	±	++	0	0	±	++++	Intermittent
3	++++	++	++++	±	+	+++	+	++++	++++	++++	Intermittent
4	++++	++++	++++	++++	++++	++++	+++	+++	±	±	Decrease
5	++++	+++	+	-	++++	++++	+++	++	++++	0	No change
6	++++	++++	±	+	++	+++	±	0	±	+	Decrease
7	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	No change
8	++++	++++	++++	++	++++	++++	+++	+++	++	±	No change (?)
9	++++	++++	+++	++++	++++	++	++++	±	±	++++	Intermittent
10	+++	++++	++++	++++	++	-	++++	++++	0	+	Decrease
11	++++	++++	++++	++++	++++	++++	++++	++++	++	++++	No change
12	++++	++	++++	++++	++++	++++	++	±	±	++++	Intermittent
13	++++	+++	++++	-	+++	+++	+++	-	-	-	No change
14	++++	++++	++++	+++	++++	++++	++++	+	+	++++	No change
15	++++	++	++++	-	++++	++++	++++	++++	++++	++++	No change
16	++++	+++	++++	-	++++	++++	+++	++++	++++	++++	No change
17	++++	++++	++++	-	++++	++++	+++	++++	++++	++++	No change
18	+++	++++	++++	+	±	+	+++	±	0	0	Decrease
19	+++	++++	++++	+++	++++	++++	+	+	±	++++	Intermittent
20	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No change
21	++++	++++	++++	++++	++++	-	+++	++++	++++	++++	No change
22	++++	++++	±	+++	+++	0	+	0	+	+	Decrease
23	+++	0	+	0	0	+	+	++	0	+	No change
24	++++	++++	++++	+	+	+	++	+	+++	0	Decrease

RESULTS

The three initial salivary lactobacillus counts in the majority of the Group A (orphanage) children were either low or negative. These cases obviously could not have a resultant decrease in the count following any type of treatment. The interest in these cases, therefore, lies in the observation of whether or not these low or negative counts will remain low or will increase following fluoride treatment in approximately the same ratio as is found among untreated, initially low-lactobacillus-count individuals.

From Table I it can be seen that only five persons (8A, 14A, 15A, 25A, 28A) had lactobacillus counts of +++ on at least one of the first three counts. Of these five, all continued to have many or all high counts following treatment with sodium fluoride. Nine individuals (2A, 3A, 6A, 11A, 13A, 17A, 19A, 21A, 27A) had low or negative counts preceding treatment. Of these, three (2A, 11A, 13A) remained low, and five (6A, 17A, 19A, 21A, 27A) rose to consistently high, or very high, counts following treatment. Subject 3A remained low most of the time, with one sporadic increase.

Table II contains the results for the controls of those children used for Table I. No individual started with very high counts, but five (1A, 10A, 12A, 16A, 23A) rose to consistently high counts during observation. One subject (10A) showing an increase was, subsequent to the increase, among those accidentally treated with sodium fluoride, and so, after treatment, must be considered among the treated subjects. In this case the count which had in-

creased before the fluoride administration did not decrease following it. Subject 18A exhibited no change in the salivary lactobacillus count prior to the accidental fluoride administration, and so may be looked upon as a control whose count did not rise during one year. Following the treatment with fluoride the count appears to have started to rise. The other subjects (4A, 5A, 9A, 20A) accidentally treated showed no sustained change in lactobacillus counts.

In Table III (treated children from the School for the Deaf) all individuals had at least one high count among the first three, but three (29, 42, 49) of the twenty-five had only one high count, and two low counts. All three showed a drop in lactobacillus counts before the sodium fluoride treatment, and continued to have low counts thereafter. It is probable that the one high count obtained was merely a sporadic increase. All of the other children continued to show lactobacilli, with six (27, 31, 36, 37, 43, 44) showing either intermittent or terminal low counts.

In Table IV, the controls for Table III, all but one individual (23) had two or more high counts among the first three taken. Subject 23 dropped after the first count, and remained low. Of the twenty-three remaining controls, six (4, 6, 10, 18, 22, 24) showed terminal low counts and five (2, 3, 9, 12, 19) showed intermittent low counts.

DISCUSSION

Although the modus operandi of fluorides in decreasing the occurrence of dental caries is not known, the chief possibilities suggested have been an increase in the resistance of enamel to decalcification by acid and an antibacterial or antienzyme action of the fluorides. If fluorides act solely by reducing acid solubility of enamel, the caries incidence could be reduced to zero without quantitatively or qualitatively altering the bacterial flora, but such an assumption has not been substantiated by scientific investigation. If fluorides act as antibacterial agents they could destroy the acidogens, in which case a critical reduction in the numbers of salivary lactobacilli would be expected; or they could alter the bacteria or their enzyme systems so as to reduce their ability to produce acid, or so that they would consume their own acid before it attacked the enamel. The recent report of Clapper[3] demonstrates that lactobacilli maintained in a medium containing 100 ppm of sodium fluoride may develop reduced ability to produce acid from glucose even when returned to a fluoride-free medium. It is also possible that the flora may be so altered as to favor bacteria which consume or neutralize acids produced by acidogens. Which of these methods, or what other method, leads to reduced caries incidence under the influence of fluorine is not known.

From still another viewpoint, the fact that lactobacillus counts were not reduced when fluorides were applied topically, although the caries activity has been reported to have been reduced in groups similarly treated, is not inconsistent. The question could well be raised whether there is any reason to expect a general reduction in salivary lactobacillus counts following topical fluoride treatment. The only really important change would be a *critical* one

from over 50,000 lactobacilli per cubic centimeter to under 1,000 lactobacilli per cubic centimeter. The maximum of 40 per cent *reduction of cavity production* in population groups is far from synonymous with *cessation of caries activity*. Whether a child develops 3 new cavities per year or 1 new cavity per year, the subject is still "caries active," i.e. the carious process still goes on. Only in the occasional individual in whom cavity production and caries activity is reduced to zero should a critical change in salivary lactobacillus count be expected.

In the data here presented it will be observed that in Group A (orphanage children) at the end of twenty-two months of study, a *rise* in the lactobacillus count was indicated for four experimental subjects and for six control subjects. None showed a decrease. In Group B (School for the Deaf children) at the end of eleven months of study, a decrease in lactobacillus count was indicated for two experimental subjects and for six control subjects. None showed an increase.

This seeming inconsistency is explained by the fact that all but two individuals (14A, 15A) in Group A had relatively low salivary lactobacillus counts at the beginning of observation, and if any significant change occurred (excepting in the two subjects with high initial counts) it had to be in the nature of an increased count. Conversely, Group B was composed chiefly of individuals selected for very high lactobacillus counts at the beginning of observation (there were six questionably high: Subjects 23, 27, 29, 32, 42, 49), so that if any significant change occurred in the counts of these subjects, it necessarily had to be a decrease.

In Group B, the fact that seven individuals among the controls ended the observation period with decreased lactobacillus counts while only two of the experimental subjects showed a decrease may be partly accounted for by the distribution of the six questionably high-count individuals in this group: five of them are in the experimental series.

The data presented for Group A are not suitable for statistical treatment. The differences observed between the experimental and control subjects of Group B are not statistically significant when the chi square test is applied.*

A study of the control subjects in both groups indicates that without purposeful treatment of any kind the salivary lactobacillus counts of a number of individuals in a group may be expected to change from high to low, or vice versa. This is an important point to remember when any treatment is evaluated by applying it to a group of selected high-lactobacillus-count subjects.

CONCLUSIONS

Topical application of 2 per cent sodium fluoride solution was not followed by any consistent post-treatment reduction in the salivary lactobacillus counts of experimental subjects. The salivary lactobacillus counts of the experimental groups which received topical applications of sodium fluoride showed no post-treatment changes that were not equally true of the control groups which had received no sodium fluoride treatments.

*Statistical evaluation was made by Dr. Gerald J. Cox, School of Dentistry, University of Pittsburgh.

Failure of salivary lactobacillus counts to be significantly reduced following topical application of sodium fluoride is not inconsistent, since only an average of 40 per cent reduction in cavity production in population groups is claimed, not a cessation of caries activity.

SUMMARY

A total of 77 children aged 7 to 9 and 14 to 16 years were divided into experimental and control groups. Each child in the experimental groups received four topical applications of 2 per cent sodium fluoride, the first preceded by an oral prophylaxis. Each control received only an oral prophylaxis. Salivary lactobacillus counts taken before, and at intervals after, the treatments showed no consistent differences, and no changes were observed in the salivary lactobacillus counts of the experimental groups that were not equally true of the control groups.

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A STUDY OF THE EXFOLIATIVE CYTOLOGY OF ORAL LEUCOPLAKIA

PAUL W. MONTGOMERY, D.D.S., M.S., AND E. VON HAAM, M.D.

From the Departments of Pathology and Oral Pathology, Colleges of Dentistry and Medicine, Ohio State University, Columbus, Ohio

IN a previous communication one of us reported a study of the exfoliative cytology of the normal oral mucosa by the Papanicolaou method.* After thus having established the criteria for the exfoliative cytology of the normal mouth, various pathologic conditions were examined by the same technic. In this article we wish to report on the exfoliative cytology of oral leucoplakia. Ten patients showing various degrees of this condition were selected for our study. The important clinical data and a gross description of the lesions of the oral cavity are listed in the following paragraphs.

CASE REPORTS

CASE 1.—A white man, aged 68. The lesion involved most of the anterior two-thirds of the dorsum of the tongue, which was smooth, white, and without fissures. The hard palate and the anterior portion of the soft palate showed the typical changes of early stages of stomatitis nicotina. There was no fissuring present in the palatal lesion. The patient was partially edentulous and stated that he had smoked a pipe for many years. He was not aware of this condition.

CASE 2.—A white man, aged 69. This patient presented disturbances of keratinization which involved almost the entire oral mucosa. The changes were most marked in the left cheek and lower left ridge. The mucosa of the cheek area showed some fissuring, and there appeared to be an irregular piling up of highly keratinized epithelium which gave the lesion a rough, horny appearance. The palate showed the changes of stomatitis nicotina. The patient gave a history of extensive pipe smoking using a very strong blend of tobacco. He was edentulous and had been aware of the condition for two years.

CASE 3.—A white man, aged 37. He had lesions involving both edentulous ridges and extending over a small portion of the hard palate. The areas were white, smooth, and showed no fissuring. The patient wore no dentures. He stated that he smoked cigarettes excessively. He was not conscious of the condition.

CASE 4.—A Negro woman, aged 39. Her lesions involved the lower left edentulous ridge in the premolar and molar areas and extended into the buccal vestibule of the premolar region. It appeared to be a rather mild form of leucoplakia and showed no fissuring. The patient smoked approximately twenty cigarettes per day. She was not aware of the condition.

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*Montgomery, P. W.: A Study of Exfoliative Cytology of Normal Human Oral Mucosa, J. D. Rec. **30**: 12, 1951.

CASE 5.—A white man, aged 50. Both cheeks, the dorsum of the tongue, the hard palate, and a portion of the lower edentulous ridge in the right molar and premolar regions were involved. The lesions were white, fairly smooth, and showed very little fissuring. The patient had been aware of the condition for approximately eighteen months. He was quite a heavy smoker. He had stopped smoking once before and the lesions had disappeared.

CASE 6.—A white woman, aged 69. Her mouth showed a small area of leucoplakia on the lower right edentulous ridge in the premolar and first molar regions. The edentulous ridge in this area showed tissue hypertrophy due to an improperly fitting denture. The patient was not aware of the condition. She does not smoke.

CASE 7.—A white man, aged 58. He had extensive lesions involving the right cheek and lower buccal vestibule areas. The areas were white, rough, and were slightly fissured. The patient had an extensive squamous cell carcinoma involving the left cheek and lower edentulous ridge in the molar region. He was a mental patient and was not aware of the existence of the leucoplakia. He gave no history of using tobacco.

CASE 8.—A white man, aged 74. His mouth showed an extensive leucoplakia of the right cheek which extended into the buccal vestibule in the third molar region. The lesion in some parts looked quite white and thickened, but was not fissured. In other portions it appeared to be in the parakeratotic stage. The patient stated that he smoked three or four cigars per day. He was not aware of the condition.

CASE 9.—A white man, aged 53. Both cheeks, the upper and lower edentulous ridges, and the hard palate were involved. The cheek lesions appeared white and smooth, while those of the ridges were thickened and fissured. The patient had known about the condition for one year. He stated that he smoked almost two packages of cigarettes per day.

CASE 10.—A white woman, aged 44. This patient had extensive leucoplakia of the right cheek, upper right alveolar ridge in the molar area, and stomatitis nicotina of the entire hard palate. The surface was roughened in some areas, but there was no fissuring. She had known about the condition for four years. The patient had smoked approximately twenty cigarettes per day for ten years.

From the foregoing clinical data we may note a number of interesting facts. The lesions involved most of the areas of the mouth that were studied in the previous survey of the normal oral mucosa.* Th cheeks and the edentulous ridges seemed to be most frequently involved in this group. The ages of the patients varied from 38 to 74 years. The group was composed of 7 men and 3 women. Seven of the group gave histories of smoking. The majority of the patients did not know that the condition in their mouths existed and apparently had no symptoms.

Smears were prepared and stained in the same manner as described by Montgomery, and differential counts of the three types of epithelial cells were done. Smears were examined from the six regions of the normal oral mucosa of these patients and from the oral lesions. These data are listed in Table I. The figures under N represent the differential counts of smears from normal areas of the mucosa and the figures under L those from the lesions. If smears were studied from two different areas of leucoplakia in the same mouth, they are designated, in Table I, L_1 and L_2.

See footnote, p. 260.

TABLE I

DIFFERENTIAL COUNTS FROM NORMAL AND DISEASED AREAS

REGION AND CELL TYPES	CASE NUMBERS AND SOURCES OF SMEARS																				NORMAL AREAS	
	1		3		4		5			6		7			8		9			10		
	N	L	N	L	N	L	N	L_1	L_2	N	L	N	L_1	L_2	N	L	N	L_1	L_2	N	L	MEAN (%)	STAND. DEV.
Soft palate																						
Blue	6	4	20		68		24			21					13		15			6		
Red	6	12	51		28		56			64					83		36			28	46.3	25.3
Yellow	88	84	29		4		20			15					4		49			66	29.9	30.0
Cheek																						
Blue	42		92		79			28	22	25			30	13	32	82	62	8		40	3	53.1	25.1
Red	50		6		16			66	44	73			16	6	68	14	38	19		59	17	44.3	25.6
Yellow	8		2		5			6	34	2			54	81	0	4	0	73		1	80	2.6	2.9
Vestibule																						
Blue	58		93		30		42			8		64			18		11			16		37.9	28.9
Red	32		5		67		55			73		35			82		89			82		56.7	27.5
Yellow	10		2		3		3			19		1			0		0			2		7.4	11.5
Tongue (ant.)																						
Blue	18		53		22		31	16		11		13			8		11			12		19.9	14.1
Red	16		5		26		53	26		41		16			68		25			58		34.2	21.7
Yellow	66		42		52		16	58		48		71			24		64			30		45.9	19.5
Tongue (post.)																						
Blue	18	8	63		32		51			5		13			32		10	4		14		26.4	19.8
Red	12	4	0		43		18			32		26			53		21	12		38		27.0	16.4
Yellow	70	38	7		25		31			63		61			15		69	84		48		46.5	20.4
Gingivae																						
Blue	9		2	21	3	0	0			2	8	0			1		0	2	3	0		2.5	3.1
Red	21		1	2	5	6	22			44	46	1			12		34	44	19	12		16.9	14.8
Yellow	70		97	77	92	94	78			54	46	99			87		66	54	78	88		81.2	15.3

A statistical comparison of the differential count of smears from normal areas in the mouths of these patients with the data from normal patients obtained in the previous study was carried out by the Statistics Laboratory of Ohio State University. The data were evaluated by means of the "t" test, which is arrived at by the following formula.

$$t = \frac{\bar{x} - \bar{y}}{\sqrt{n_x s_x^2 + n_y s_y^2}} \cdot \sqrt{\frac{(n_x + n_y - 2)\, n_x n_y}{n_x + n_y}}$$

$\bar{x}$ and $\bar{y}$ denote the means of percentage distribution, n_x and n_y the number of counts in both groups, and s_x and s_y the standard deviations of the percentage distribution. High t values represent significant differences between the means of both groups. They are marked by one asterisk in Table II. Those values marked by two asterisks suggest that a more extensive experiment might lead to significant differences in these means. The second patient of our series was omitted from the statistical analysis because almost the entire oral mucosa was involved and no smears could be obtained from normal mucosa.

TABLE II

T VALUES OF PERCENTAGE DISTRIBUTION OF CELLS IN NORMAL AND DISEASED AREAS

AREA	CELL TYPES	MEAN VALUE NORMAL AREA	MEAN VALUE LEUCOPLAKIA	T VALUES
Soft palate	Blue	50.8	23.9	2.43*
	Red	41.7	46.3	− .43
	Yellow	7.5	29.9	−3.83*
Cheek	Blue	51.5	53.1	− .16
	Red	43.8	44.3	− .05
	Yellow	4.7	2.6	.46
Vestibule	Blue	45.0	37.8	.71
	Red	42.9	56.7	−1.47**
	Yellow	12.1	7.4	.64
Tongue (ant.)	Blue	19.7	19.9	− .03
	Red	47.3	34.2	1.80**
	Yellow	33.7	45.9	−1.64**
Tongue (post.)	Blue	30.9	26.4	.75
	Red	35.5	27.0	1.53**
	Yellow	33.5	46.5	−2.31**
Gingivae	Blue	7.3	2.5	1.56**
	Red	17.9	16.9	.14
	Yellow	75.0	81.2	− .84

From the analysis of the t values we may conclude that smears from the normal areas of the mucosa in our patients with leucoplakia have the tendency to show fewer blue cells and more yellow cells in the palate region and more yellow cells in the posterior tongue region than the corresponding areas of normal subjects. Chi square tests were used to compare the cellular distribution in normal and diseased areas. Only 6 of the 9 cases showed a significant deviation from the normal distribution. In 5 of these 6 cases the diseased areas had more yellow and fewer red cells than the normal areas.

A cytologic study of the individual smears from the lesions and the normal oral mucosa of patients with leucoplakia did not add much to the knowledge gained by the statistical analysis of the cell counts. No typical "leucoplakia cell" could be discovered and even detailed cytologic study failed to reveal any abnormality in the nucleus or nucleolus suggestive of any change in the cell morphology. The yellow-staining cells found in such increased number in smears from the lesions were mostly without nuclei and did not differ from similar cells found in gingival smears of normal individuals. However, their appearance in leucoplakia of the cheek and the palate made the smears of these lesions quite different from the smears of the normal mucosa in these regions. A more generalized disturbance of the process of keratinization beyond the scope of the actual lesion could also be recognized in the cheek smears from patients with leucoplakia, as these showed a predominance of red cells when compared with cheek smears of normal patients. However, this difference was not forceful enough to have any statistical or diagnostic value. A study of the bacterial flora and of the other cellular elements present showed no noteworthy changes.

SUMMARY

Cytologic smears were studied in 10 patients with oral leucoplakia, both from the lesions and the six normal areas of the mucosa.

Smears of normal and pathologic areas of patients with leucoplakia showed some evidence of increased keratinization. This evidence was most marked in smears from the lesions themselves and in some cases was striking enough to be of diagnostic value. No cytologic characteristics were found suggesting premalignant or malignant changes.

THE EFFECTS OF DENERVATION AND ISCHEMIA UPON THE TEETH OF THE MONKEY

EARL O. BUTCHER AND A. CECIL TAYLOR

Departments of Anatomy, College of Dentistry and the Graduate School of Arts and Science, New York University, New York, N. Y.

THIS investigation was undertaken to determine the effects of denervation and ischemia upon the teeth and to study the regenerative potentialities of pulp tissue and of connective tissue into the pulp cavity. Justification for investigation of this problem arose from the frequent inquiries regarding the effects of cutting the inferior alveolar nerve in cases where resection of the mandible was made and where there was interference with the nerves and vessels in the Caldwell-Luc operation.

King[1] removed a portion of the nerve in the mandibular canal of dogs. This caused little effect on the teeth other than a modification of their position. Resection of the cervical sympathetics in the rabbit resulted in accelerated growth of the incisors for only a few days. He was inclined, as was Leist,[2] to attribute the increased eruption rate to vasodilation. Edwards and Kitchen[3] found that resection of vasoconstrictor (sympathetic) fibers supplying tooth germs in four kittens accelerated growth from 2 to 15 per cent; resection of afferent (somatic) fibers of inferior alveolar nerve had no definite effect on rate of tooth development.

Resection of the inferior alveolar nerve in rats (Taylor and Butcher[4]) resulted in a 30 per cent greater eruption rate of the incisor. Removal of the cervical sympathetic chain and ganglia and transection of the common carotid failed to alter the eruption rate. However, when the inferior alveolar nerve was later destroyed in these same animals, then the 30 per cent increase of eruption rate followed. This led to the conclusion that the effects of resection of the inferior alveolar nerve were not via the sympathetics.

No investigators have followed the effects of inferior alveolar nerve resection for a very long period in an experimental animal. Accordingly, there seemed justification for seeing if there were effects on the gingiva, eruption, a greater caries incidence, changes in the pulp, changes in the hardness of the dentin and enamel, etc., after resection of the inferior alveolar nerve in the monkey.

Ischemia of the tooth was to be investigated to see how it would affect the tooth pulp. One method of accomplishing this seemed to be by strangulation. Orthodontists seemed uncertain if a tooth pulp could be strangulated and indefinite about the fate of the pulp if the pulp vessels were strangulated for an interval. Strang[5] stated that sudden tension on a tooth by excessive force may

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so injure the blood vessels entering the pulp chamber as to cause serious venous congestion, blood stasis, and finally death of the parts. According to him, too powerful force used in tooth depression may shut off the arterial supply of the pulp tissues and thus produce a devitalization of the various elements in this organ. Whether or not there is restitution of the pulp, if it is killed, has not been determined.

In the rat, Taylor and Butcher[4] found that retraction caused necrosis of the pulp tissues. Upon removal of the retraction, tissue regenerated into the pulp cavity but it was devoid of odontoblasts and dentin-forming potentiality. The odontoblasts seem to be very specialized cells which cannot differentiate from all connective tissue cells.

Another method of testing the effects of ischemia on the pulp was by performing apicoectomies. In addition, apicoectomy will deprive the pulp cavity of nerves for an interval and make possible the study of their regeneration into the pulp cavity. In fact, apicoectomy experiments will show to what extent connective tissue outside of the tooth will replace pulp tissue. Boyle[6] reviews some very interesting cases where the pulp remained vital after the roots were fractured in human beings. The events following apicoectomy have not been followed carefully in experimental animals. Bauer[7] has shown by experiments on cats and dogs in which he resected the root ends of the teeth that in a few months after the operation the cut surfaces of the dentin were covered with newly deposited cementum and a new periodontal membrane. Davis[8] observed in human teeth that a repair of the pulp took place and osteoid material was deposited in it, provided the point of excision down the pulp canal was not beyond the apical foramen.

In these present investigations it is proposed to determine the effects of apicoectomy on noninfected teeth. The endodontist, on the other hand, is confronted with an infected apex and in many instances a diseased pulp. In the latter instance the practice has been to fill the canal and perform an apicoectomy. If our investigations show that the distal pulp will survive after removal of the apical portion of the tooth, then possibly the root canal therapist might save some pulps by our method. With a larger foramen, it is entirely possible also that there would be an invasion and clearing away of the necrotic tissue, if any existed, in the pulp and the migration of vital tissue into the pulp cavity.

Experiments on the effects of denervation and ischemia upon the teeth may suffice to give us information which will help to evaluate and determine clinical procedure in many instances.

EXPERIMENTAL PROCEDURE

The best method of studying the effects of denervation of the teeth without interfering with their vascular supply appeared to be the transection of the nerve proximal to the mandibular foramen. Not only was transection necessary but the central nerve stump had to be deviated in such a way as to prevent regeneration.

Monkeys (Rhesus) either with permanent or deciduous dentition were used in the denervation experiments. Those with deciduous dentition at the time of operation were used to see the effect on eruption and hardness of the permanent teeth while older monkeys with permanent teeth presented a better opportunity of studying the effects on caries, the gingiva, and wear.

Sodium pentobarbital was injected intraperitoneally as anesthesia and 2 c.c. usually sufficed to keep an 8-pound monkey quiet for the operative period of about 1 hour.

An area of skin posterior to the ramus of the mandible was then depilated and carefully cleaned. An incision about ½ inch long was made parallel to the posterior border of the ramus of the mandible. Parotid gland material was turned anteriorly, and the fascia was separated until the posterior border of the internal pterygoid muscle was apparent. By deep dissection between the internal pterygoid and the ramus of the mandible the sphenomandibular ligament and about ¼ inch of the inferior alveolar nerve were exposed. In some instances the posterior fibers of the internal pterygoid muscle had to be transected for working space. No ill effects were noticed from this following the operation.

In the first experiments, ligatures were tied at each end of the exposed portion of the inferior alveolar nerve and then the nerve was transected between the ligatures. Subsequent observations after the sacrificing of a few animals showed regeneration of the nerve fibers around the ligatures into the mandibular foramen. Thereafter, in other animals the nerve was cut close to the mandibular foramen and the proximal end was sutured posteriorly and laterally to dense parotid fascia or to the external pterygoid muscle.

Various methods of depriving the teeth of their vascularity for intervals were attempted. Ligation of the inferior alveolar and mental arteries sufficed to eliminate the blood for only a short interval as shown by exposure of the vessels in the pulp. Orthodontic appliances anchored on adjoining teeth so as to retract the tooth into its alveolus were also ineffective. By running rubber bands through small holes near the incisal borders of the lower first incisors and attaching their ends to a wire passed circumferentially around the mandible at the symphysis, the incisors were retracted into their sockets with the force of about 250 grams. Teeth with large and small apical foramina of different monkeys were investigated. One incisor was extracted after an interval of 10 to 16 days, and at the same time the other was released from tension. The released tooth, left intact for varying periods, was then extracted and prepared histologically. Ischemia was accomplished by this procedure in some instances and it permitted a study of the fate of the pulp deprived of vascularity and its regenerative potentialities.

To further investigate the effects of ischemia and denervation on the contents of the pulp chamber, either the apex, the apical third, or the entire pulp (via a window in the apical third) was removed. It seemed necessary to cut away the apical third of the tooth in some instances since, according to Hatten, Skillen, and Moen,[9] there are often small accessory canals in the apical third of

the tooth. These operations were usually performed on the upper first incisors. They were accomplished easiest via an external approach since the apices of the teeth were located deep and superiorly. The skin incision was made just superior to the external naris. Bone and apex removal were accomplished with dental burs. At intervals following the operation, extracted teeth or in some instances portions of the jaw with intact teeth (from sacrificed animals) were prepared histologically. Such operations accomplished denervation, ischemia, and permitted a study of pulp regeneration.

OBSERVATIONS

Resection Experiments

In the first 4 monkeys, ligatures were tied at the ends of the exposed portion of the inferior alveolar nerve and the nerve was transected between the two ligatures. After the unilateral resection, healing was rapid and apparently no difficulty was experienced in eating. At intervals, the teeth were radiographed, examined for caries, for wear, and for gingival disturbances, the resected side being compared with the side having the nerve intact. Monkeys whose permanent teeth were in the process of formation were carefully observed to see if the rate of eruption of the two sides was different.

Three monkeys were sacrificed 3, 6, and 8 months after the operation and the resected nerve was fixed in Bouin's fluid and later stained by the Bodian method. The jaws with intact teeth were cut into small pieces, fixed in various fixers, and later sectioned and stained.

In all 3 monkeys no gingival disturbances were noted on the resected side. In 1 monkey the lingual cusps of the molars of the resected side were slightly more worn. Roentgenograms showed no differences in the rate of eruption between the 2 sides and caries incidences were equal. Several clinicians carefully examined the teeth and roentgenograms. They were unable to note any differences between the jaws and unable to identify the jaw whose nerve had been transected.

Stained sections of the nerve showed that a few fine fibrils had regenerated across the site of the transection into the mandibular foramen. Upon careful examination of the teeth sections, fine nerve fibers, probably with a sympathetic function, were present in the pulp but the nerve content in comparison to the pulp of the teeth of the unoperated side was greatly reduced. Other than this difference in the nerve content of the pulp, no other changes were found.

The remnants of the nerve, distal to the transection, of the fourth monkey, killed 6 months after the operation, were histologically prepared and examined. This nerve contained few, if any, fibrils. Grossly, the operation caused no changes in the teeth or gingiva and there were few nerves in the sections of the teeth.

In view of the fact that the resected nerve regenerated across the point of resection and the ligatures and the possibility that an interval of 8 months was not long enough for any modifications in the teeth after resection, further experiments seemed advisable. The inferior alveolar nerve was exposed uni-

laterally in 5 monkeys. They were resected closely to the mandibular foramen and the resected end was sutured in most instances into the pterygoid muscle. These monkeys were again carefully examined at intervals to see if there were any greater caries incidence, wear, effect on eruption, or gingival disturbances in the resected side. Two of these have been followed for 1 year while the others were sacrificed at 4, 5, and 6 months after the operation. No gross changes appeared as the result of the resection. In the sacrificed animals the proximal end of the resected nerve, firm and often bulbous due to its growth, was still securely sutured to the external pterygoid muscle. The distal end, extending into the mandibular canal, on the other hand, was much smaller in diameter, and softer.

Histologic preparations of the contents of the mandibular canal of the resected side showed a few fine fibrils, probably with a sympathetic function (compare Figs. 1 and 2). The teeth, with the exception of a diminished nerve content in the pulp, appeared the same as the prepared teeth from the side with the nerve intact.

Hardness of the enamel and dentin was determined with the Knoop indenter.* A 500-gram load was used on the indenter in all cases. Each value given in Table I is an average of many indentations, the plus or minus values being an average deviation. The data do not show any evidence of a significant difference in hardness between the teeth of the denervated jaw and the opposite jaw with the nerve intact.

TABLE I

INDENTATION HARDNESS (KNOOP METHOD)

MONKEY	TOOTH	DENERVATED		INTACT NERVE	
		DENTIN	ENAMEL	DENTIN	ENAMEL
5	Second molar	61 ± 3	282 ± 14	62 ± 3	282 ± 6
5	Canine	54 ± 4		59 ± 2	
7	Second molar	63 ± 1	252 ± 11	64 ± 2	251 ± 21
7	Canine	61 ± 2		55 ± 3	

Retraction Experiments

Lower first incisors with large apical foramina were retracted by the circumferential appliance in 7 monkeys. The appliance appeared to cause no discomfort. Many incisors became discolored. The retractive force at the beginning of the experiment was about 250 grams and 10 to 16 days later it had decreased to 80 to 150 grams. The appliance was removed at the end of 10 to 16 days and the teeth were very loose. One tooth was then extracted in each monkey and histologically prepared.

The extracted teeth of all the monkeys indicated that the root ends had been turned inward toward the pulp by the retractive force (Fig. 3). The pulp at the apex was compressed together and was very dense. Strangulation of the blood vessels had occurred, resulting in the degeneration of the distal pulp and the disappearance of all odontoblasts (Fig. 3). The distal pulp consisted of a few congested vessels and distorted cells and fibers.

*We are indebted to Dr. George Dickson of the Dental Research Laboratory of the National Bureaus of Standards for these determinations.

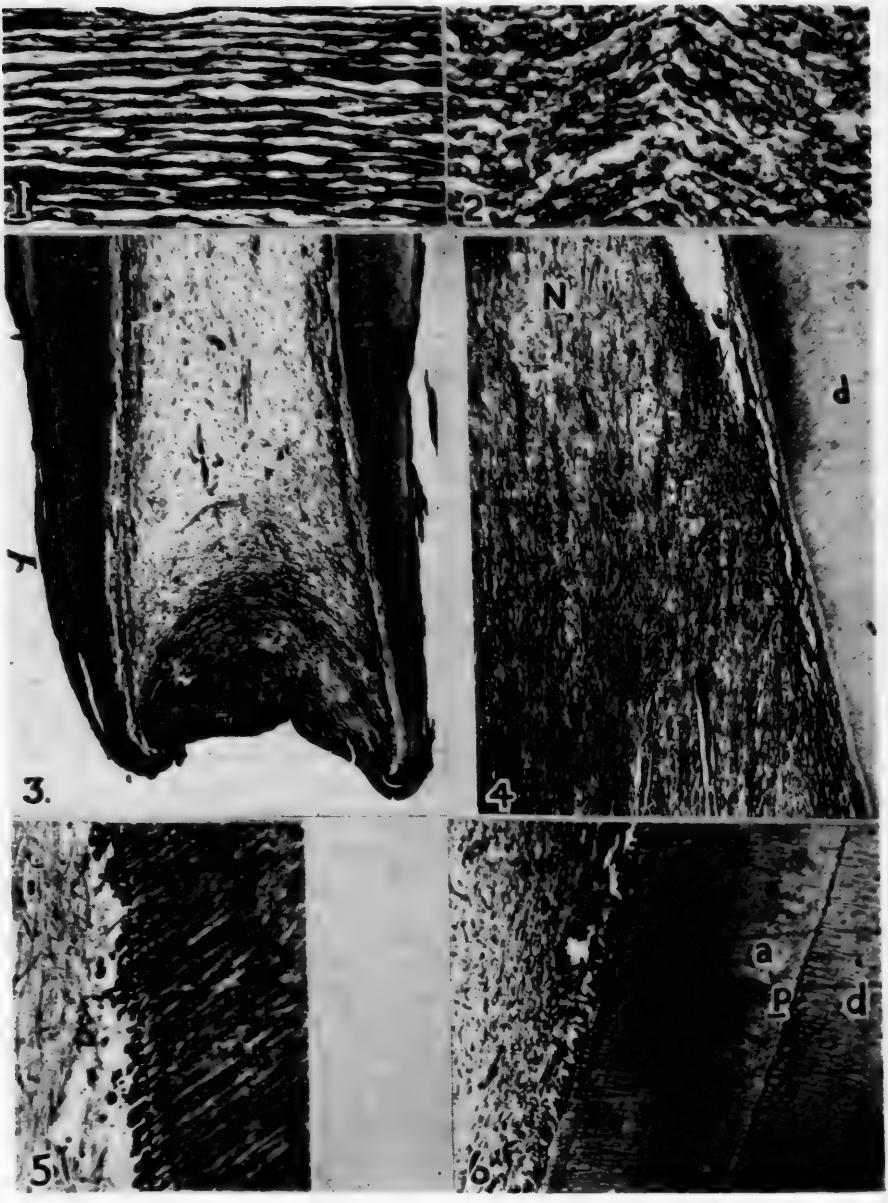

Fig. 1.—Normal nerve prepared with Bodian's technic.

Fig. 2.—Nerve taken distal to resection 6 months after the operation.

Fig. 3.—Tooth with large apical foramen. Retracted for 16 days. Note the avascular and necrotic pulp distally, and the compressed pulp proximally.

Fig. 4.—Tooth was retracted for 16 days, then released for 11 days. Necrotic pulp (*N*) is being invaded by cells distally while fibers have appeared among cells proximally. *d* = Dentin.

Fig. 5.—Distal portion of tooth which had been released for 22 days. Processes of cells are directed toward and in some instances into dentinal tubules.

Fig. 6.—Tooth released for 60 days, showing atubular (*a*) dentin deposited internal to predentin (*p*) formed prior to retraction.

The other incisors in the 7 different monkeys became firm without any treatment. They were extracted and prepared histologically at intervals after their release. Study of a tooth 11 days after releasing showed that the apical half of the necrotic pulp had been invaded by cells of varying shapes from the compressed area of the apex (Fig. 4). Among most of the cells, except those at the very distal extent of their invasion, were fibers whose density increased toward the apex. The necrotic pulp appeared to serve as a medium into which the cells and fibers grew. Invasion of the pulp, thus, progressed as a wave from the apex to the distal end of the tooth. While growth was progressing distally, flat cells were lining up adjacent to the predentin. In 1 monkey's tooth which had been released for 22 days, the cells next to the predentin in the apical region had become enlarged, the nuclei were vesicular, and the cells resembled osteoblasts. A narrow zone of structureless material had been deposited internal to the predentin. In the distal portion the cells were round, contained small, dense nuclei, and processes of the cells had become directed toward and in some instances into the former dentinal tubules (Fig. 5).

In 2 different monkeys whose teeth had been released for 44 and 60 days, respectively, odontoblasts had differentiated and dentin had been deposited directly on the dentin formed prior to the retraction. In the distal areas, other than a bend in the dentinal tubules, there was little indication where the process was renewed; while in the apical portion of the tooth, small areas of predentin occasionally persisted and interrupted the continuity of the dentinal tubules. In preparation of the teeth, splits in the sections frequently occurred where the renewed growth joined the material deposited prior to retraction. The thickness of the newly deposited dentin, great at the apex, became thinner distally.

In 2 other monkeys whose teeth had been released for 60 and 70 days, structureless dentin had been deposited internal to the predentin in the apical half of the tooth. Since this band was narrow, the duration of this process had been short. Tubular dentin was then formed. The sequence of these events is shown in Fig. 6. The band of atubular dentin was irregular, became thinner distally, and disappeared entirely at approximately the mid-point of the length of the tooth. At this point tubular dentin had been deposited directly internal to the dentin formed prior to the retraction of the tooth.

In the seventh monkey whose tooth was extracted 60 days after releasing and prepared, cementum had been deposited internal to the dentin in the apical half of the tooth (Fig. 7) while distally no deposition of any kind had occurred. In this tooth for some reason no odontoblasts were formed. Their lack appeared not to be entirely related to the amount of retractive force for in other instances where odontoblasts regenerated, the retractive force had been as great.

The type and amount of material formed in these teeth with large apical foramina after release had thus varied. Frequently, in the apical half of the tooth, a variable quantity of either structureless dentin or cementum was deposited internal to the predentin. Internal to this, except in the case of cementum, tubular dentin was deposited. In the distal half of the tooth no

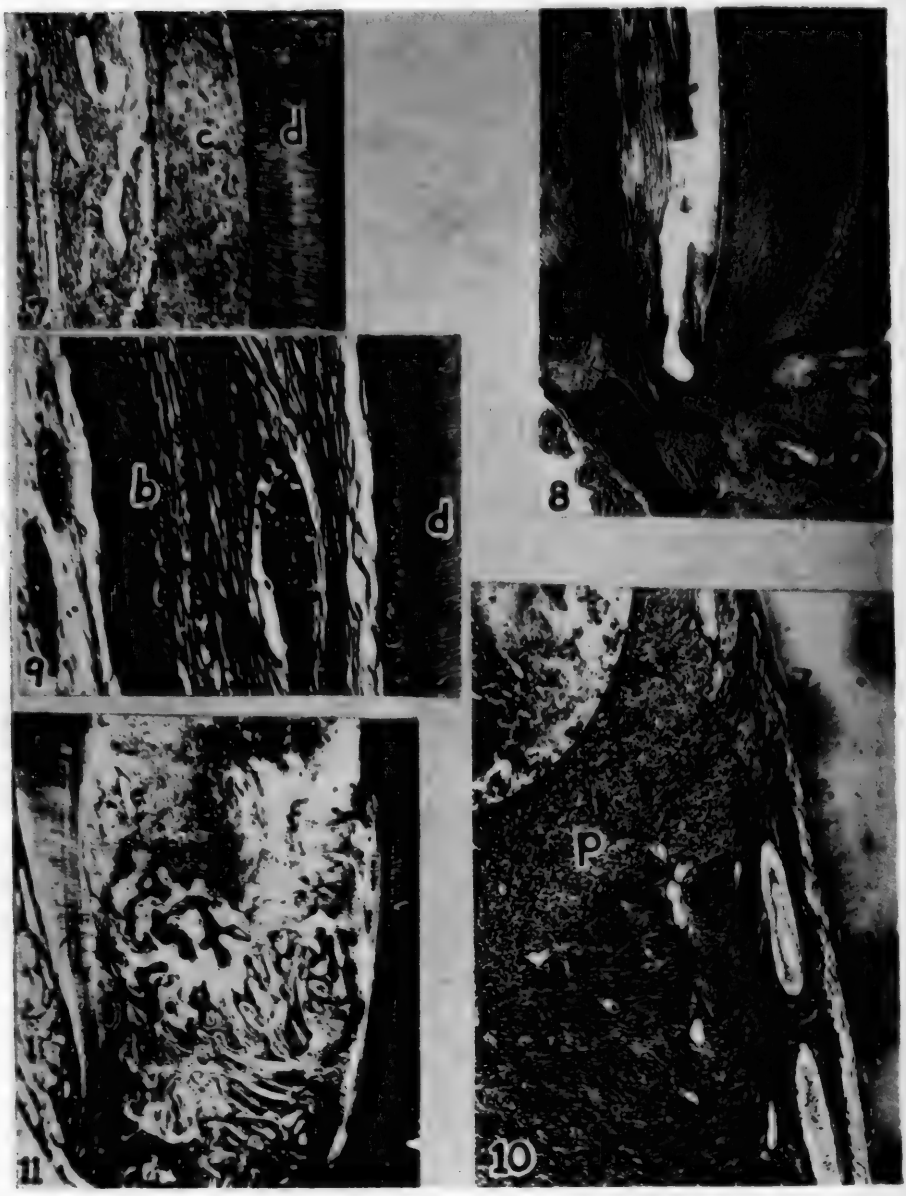

Fig. 7.—Tooth released for 60 days, showing cementum (*c*) deposition internal to the dentin in the apical region.

Fig. 8.—Root of tooth with small foramen retracted for 11 days.

Fig. 9.—Photograph shows bonylike tissue (*b*) in the pulp of a tooth apicoectomized 77 days previously.

Fig. 10.—The pulp of this tooth was removed 18 days previously. Phagocytes (*p*) have invaded the clot followed by the invasion of connective tissue into the pulp cavity.

Fig. 11.—Forty-seven days previously the pulp of this tooth was removed. Spicules of bone have now filled the apical portion of the tooth.

such formation of structureless dentin was ever observed. Instead, processes of cells oriented themselves and invaded the former dentinal tubules and tubular dentin was formed. A bend in the dentinal tubule in many instances was the only indication where the process had been renewed.

Incisors with small apical foramina were retracted in 4 monkeys. When the teeth were released and 1 was extracted and sectioned from each monkey, it was observed that the pulp had not been destroyed. This result was surprising for these teeth were expected to be more easily destroyed by the retractive force than teeth with large apical foramina.

The pulp was not compressed together and the vessels were not strangulated in the tooth with a small foramen because it was more protected by the thicker root ends and the more secure anchorage of the tooth in the alveolus (Fig. 8). The apex of the tooth in Fig. 8 shows some resorption.

Apicoectomy and Pulpectomy

These experiments were usually performed on the upper first incisors and fall into 4 groups: apicoectomy of teeth with small apical foramina; apicoectomy of teeth with large apical foramina; removal of a window in the root region; and pulpectomy via a window in the apical third.

Cementum deposition at the apex and into the pulp cavity for varying distances followed apicoectomies of teeth with small apical foramina. In extracted incisors which had been apicoectomized 140 days previously, shelves of cementum had been formed at the apex until they nearly sealed off the pulp tissue in the pulp cavity. The pulp consisted of dense tissue, masses of white blood corpuscles, and occasional blood vessels. All predentin had disappeared. In some regions resorption of the dentin had occurred, resulting in great excavations. Neither odontoblasts nor islands of bony tissue were encountered in the pulp.

Where apicoectomies of teeth with large apical foramina were performed, connective tissue regenerated into the pulp cavity. Fig. 9 shows such a regeneration 77 days after the operation. Islands of bonylike tissue resembling cementum surrounded by osteoblasts are present. There had been no cementum formed internal to the predentin except in the very apical region. Predentin still persisted distally and in many regions cells had invaded it. No odontoblasts were present. The pulp consisted of a very loose connective tissue, the vascularity of which varied in different teeth. The few existing nerve fibers (seen in sections stained with Bodian's method) were closely associated with the blood vessels.

When a 2 mm. oval hole was made through the cementum and dentin in the apical third of the tooth, extensive migration of periodontal tissue into the hole took place. Large quantities of cementum were then formed in repairing the hole and masses of cementum protruded into the pulp. Internal to the cementum was usually found a layer of structureless dentin.

Teeth were extracted and histologically prepared 18 to 50 days after the removal of the pulp through an oval opening in the apical third of the tooth. In teeth taken 18 days after the operation, phagocytes had proceeded distally

in their invasion of the clot and they were closely followed by invading blood vessels and connective tissue (Fig. 10). An occasional island of bony tissue surrounded by osteoblasts was found. Fig. 11 shows the extent of the repair 47 days after the operation. Repair had progressed distally, and many bone spicules have been formed in the pulp. No odontoblasts had appeared and no dentin had been deposited. Since dentin had not been formed in this interval, it is doubtful if it ever would form. The pulpectomy had thus destroyed the formative tissue supplying odontoblasts and dentin.

DISCUSSION

The purpose of this investigation was to determine the effects of denervation and ischemia upon the teeth. Experiments used in making the investigations also enabled us to study the effects of retraction on the pulp and to study the survival and regenerative capacity of the pulp.

Development of teeth and maintenance of tooth structure do not depend on innervation as shown in the present experiments. It is true that the monkeys were followed for only 1 year but this represents at least 4 or 5 years in terms of human life. From our observations and experiments ischemia, except vascular disturbances at the apex of the tooth, would seldom be established to the extent of permanently injuring a tooth. Adequate vascularization is quickly re-established through collateral circulation.

There has been little or no study of the effects on the pulp when the tooth is retracted. In fact, whether or not teeth could be retracted seemed debatable until rather recently. Gottlieb and Orban[10] maintained that depression of teeth was possible and referred to the resorption at the base of the alveolus. The work of Lefkowitz and Waugh[11] showed that teeth may be depressed in their alveoli in the dog and that there was resorption along the walls of the alveolus. In the present studies when the apical foramen was extensive and the tooth was less securely anchored in the alveolus, the blood vessels at the apex became compressed and the pulp, except that at the very apex, degenerated. After release from the retractive force sufficient tissue with odontoblastic and dentin-forming potentialities had survived at the very apex to repopulate the pulp cavity with odontoblasts and to deposit dentin. The difference in the type of material deposited in the apical and distal regions of the pulp after retraction in many instances, that is, the formation of an intervening layer of structureless dentin between the predentin and dentin in the apical region, is hard to explain. Whether the entrances into the dentinal tubules are obliterated or some other change such as lack of orientation occurs which is not conducive to the deposition of tubular dentin has not been determined at this time.

Lefkowitz, Bodecker, and Shapiro[12] showed that histodifferentiation of odontoblasts occurred only when ameloblasts were present. Following papillectomy, histodifferentiation of fibroblasts into odontoblasts did not occur. The apicoectomies in the present experiments sufficed to show that the area which has odontoblastic and dentin-forming potentialities is restricted and that these cannot be derived from all connective tissue. In view of Avery's observations[13]

it seems quite possible that odontoblasts are not derived from fibroblasts but from special cells, probably of neural crest origin, and according to the present investigations these special cells are restricted to the immediate apical area.

CONCLUSIONS

1. Teeth develop and maintain normal structure without a nerve supply.

2. Unless the disturbance of blood vessels is in the apical region of the tooth, ischemia could seldom be established to the extent of causing permanent injury.

3. The pulp of teeth with large apical foramina are more easily injured by retraction than the pulp of teeth with small foramina. In the case of teeth with large foramina, odontoblasts and most of the pulp degenerate upon retraction; upon release, these are replaced from compressed tissue at the apex.

4. Following apicoectomy, invasion of the pulp cavity with connective tissue occurs. No odontoblasts regenerate but osteoid tissue is deposited, surrounded by osteoblasts. Tissues possessing odontoblastic and dentin-forming potentialities appear to be quite specialized and restricted to an area at the apex. When not destroyed, these tissues can supply new odontoblasts and dentin in a regenerating pulp.

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MODIFICATIONS OF MANDIBULAR ARCHITECTURE FOLLOWING REMOVAL OF TEMPORALIS MUSCLE IN THE RAT

SIDNEY L. HOROWITZ AND HARRY H. SHAPIRO

Research Department, School of Dental and Oral Surgery, and Department of Anatomy, College of Physicians and Surgeons, Columbia University, New York, N. Y.

INTRODUCTION

DESCRIPTIVE studies of bone in the nineteenth century emphasized the importance of extrinsic factors in skeletal development. The Cullman-Meyer hypothesis,[1] and the concepts of Wolff,[2] explaining the formation of bone and its internal arrangement, stress the factor of functional stimuli. These theories were based primarily upon observations of the long bones, especially the human femur, which Koch[3] later analyzed mathematically to demonstrate a relationship between stress and structure. The appearance of an adult type of internal architecture in the human femur, following the imposition of the forces of weight bearing and walking in early childhood, has also been reported.[4]

Benninghoff[5] described lines of force, or trajectories, in the trabeculation pattern of the human mandible which, he stated, conform to the forces exerted on the jaw by skull and masticatory musculature. Experimental methods in the investigation of bone formation[6-16] have permitted a study of the dynamic relationship between alterations of function and bone architecture.

The genetic determination of form and structure in bone has been demonstrated experimentally by Fell and Robison,[6] and Murray and Selby.[7] Intrinsic rather than extrinsic factors have been shown to be of greater importance in the early stages of skeletal differentiation whereas extrinsic factors are more important as the skeletal form is refined. Pottorf[8] and Howell[9] have shown that normal functional forces influence growth of the long bones in diameter rather than length, and in the diameter of the trabeculae internally rather than the direction which they assume. Deformities of the maxillae and mandibles of experimental animals have been produced by Baker[10, 11] who altered masticatory function by unilateral extraction of teeth.

Other investigators have modified masticatory function in various ways to produce skeletal changes. Pratt[12] found extreme differences in the vertical proportions of the mandibular ramus following experimental removal of the masseter muscle. In his experiments on skull form in the rat, Washburn[13] produced cranial asymetries by unilateral section of the seventh nerve. In another study,[14] he found that partial removal of the zygomatic arch had no effect on skull form unless the function of the masseter muscle was affected.

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Our object in this experiment has been to analyze modifications of external form and to study changes in the internal architecture of the mandible resulting from alteration of masticatory function following unilateral removal of the temporalis muscle in the newly weaned rat.

MATERIALS AND METHOD

Eight male Sherman strain rats from 2 litters were used as experimental subjects; additional animals from each litter served as unoperated controls. Each animal was about 30 days of age at the time of operation; the average weight was approximately 50 grams. Anesthesia was induced with intraperitoneal injections of pentobarbital HCl, 3 mg. per 100 grams of body weight.

The skin was incised from above the orbit to the retro-auricular area on the right side. A second incision, at right angles to the first, was extended to the inferior border of the mandible. The skin and superficial tissue were retracted and the right temporalis muscle and part of the masseter muscle exposed.

A. *B.*

Fig. 1.—*A*, Gross specimen with skin removed. View, from above, of skull of an experimental animal; the right temporalis had been removed at the age of 30 days, and animal sacrificed 173 days, postoperatively. Temporalis muscle and linea temporalis are absent on the operated side.

B, Anterior view of an experimental animal; right temporalis muscle was removed at the age of 32 days, and animal sacrificed 181 days, postoperatively. Upper incisor teeth deviate to side of operation.

The temporalis muscle was dissected from linea temporalis, and a portion of the muscle inferior to the line of origin removed. The mandible was manipulated to localize the insertion of the temporalis muscle on the coronoid process, and the exposed periosteum of the skull and coronoid process removed, care being taken not to damage the underlying bone. The medial portion of the zygomatic arch was also exposed and some upper fibers of the masseter muscle removed. Silk skin sutures and collodion were used to close the wound. The animals were then allowed to mature, subsisting on an ordinary laboratory diet, and were sacrificed at intervals. Gross examination was made by dissecting away the overlying skin and exposing the musculature of the skull.

Grenz ray examination was employed to compare and analyze differences between the mandibles of the control, operated, and unoperated sides of the mandibles of the experimental animals. The specimens were exposed to grenz rays for approximately 45 seconds each, at 9000 volts and 10 amperes, using Kodak spectroscopic plates of type V-O.[15]

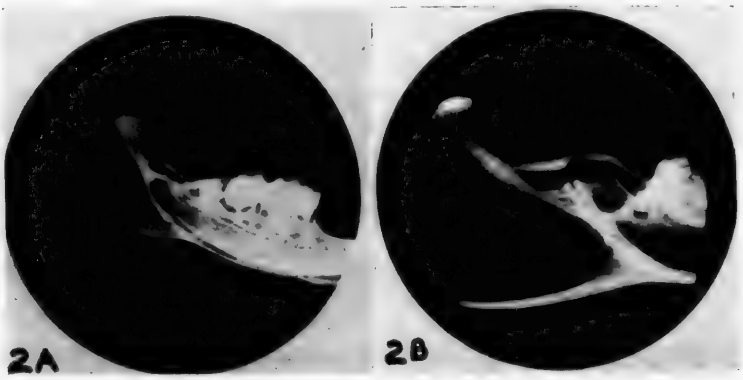

Fig. 2.—*A*, Grenz ray of right side of mandible of a normal 30-day-old rat. Coronoid process and condyle are well differentiated. Trabeculation pattern is not well organized.

B, Grenz ray of right side of mandible of a normal control animal sacrificed when 240 days of age. Proportions are similar to those of mandible at 30 days, but trabeculation pattern is definitely organized.

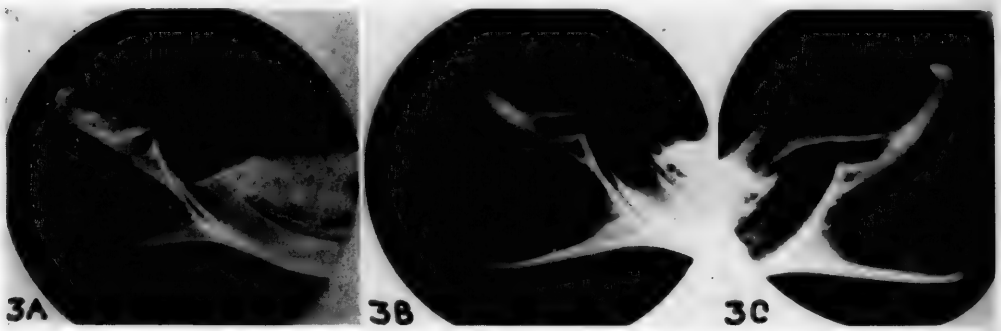

Fig. 3.—*A*, Grenz ray of right side of mandible of an experimental animal; right temporalis muscle was removed at the age of 32 days, and animal sacrificed 51 days, postoperatively. Coronoid process is absent, and changes in form and direction of head and neck of condyle are apparent.

B, Grenz ray of right side of mandible of an experimental animal; temporalis muscle was removed at the age of 30 days, and animal sacrificed 173 days, postoperatively. Coronoid process is absent, and form and direction of head and neck of condyle have undergone modification. There is a triangular area of decreased trabeculation posterior to apex of the incisor tooth; trabeculation pattern is generally less well developed than in normal control.

C, Grenz ray of left (unoperated) side of mandible of same animal shown in *B*. Internal architecture and outer form are comparable to that of normal control. There is a triangular area of decreased trabeculation posterior to apex of the incisor tooth, but not so marked a change from the normal as in *B*.

DISCUSSION

Although the coronoid process and linea temporalis were well differentiated at the time of operation, no evidence of these structures could be seen after a period of less than 2 months following the operation (Figs. 1, *A* and 3, *A*).

The intrinsic nature of the form of the mandible and the severe effects resulting from the absence of functional stimuli to that part of the mandible providing a site of insertion for the temporalis muscle have been substantiated. These findings are in agreement with those of Washburn,[16] who performed similar experiments on newborn rats.

Examination of the grenz ray studies of our specimens indicates that modifications of internal architecture of the mandible of the operated side are consistent with loss of functional stimuli. The trabeculation pattern shows alterations as compared with the control mandible; the form and direction of the head and neck of the condyle are modified.

An additional change in the internal architecture of both the operated and unoperated sides of the mandibles of the experimental animals is noted in a triangular area of diminished trabeculation posterior to the apex of the incisor tooth. This finding is more marked on the operated side, but is visible as a deviation from the normal on both sides of the mandible in the experimental animals.

SUMMARY

1. The temporalis muscle was removed unilaterally in a number of 1-month-old rats.

2. Modifications of outer form and internal architecture of the mandible resulted.

3. Gross examination of the operated animals showed no evidence of the coronoid process, site of insertion of the temporalis muscle.

4. Changes were noted in the form and direction of the neck and head of the condyle on the operated side.

5. Modifications of internal architecture were revealed by grenz ray examination.

The authors are indebted to Dr. Edmund Applebaum for his aid in carrying out the grenz ray studies reported in this paper.

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DIMENSIONAL CHANGE OF AGAR-AGAR GEL ON IMMERSION IN VARIOUS SULFATE SOLUTIONS

E. A. LUSTER, A.B.

Louisville, Ky.

INTRODUCTION

IN RECENT years, the use of agar-agar as an impression material has gained favor in the dental profession. It is far from a perfect material for this purpose, but much superior to materials used previously.

Inasmuch as there is a definite volumetric change in agar-agar with time and conditions, it has been the custom in dental offices to immerse the impression in a 2 per cent solution of potassium sulfate. It has been claimed that this solution will stabilize the agar gel against dimensional change. The following work is an attempt to determine by physical methods the effect of different concentrations of potassium sulfate on a specific agar gel as compared to the effect of water and exposure to air on the same gel concentration through various time intervals. Several metal sulfates in a 2 per cent concentration in water have been tried also. Sulfates were chosen inasmuch as the die material sets very well in sulfates, whereas other anions might interfere with setting, forming a surface difficult or impossible to measure by the methods of this paper.

PRELIMINARY WORK

A gross experiment was performed to determine if there was a definite penetration of salt solution into the agar gel under the conditions of the test.

A quantity of potassium ferrocyanide was incorporated with the agar material. After cooling, a piece of this prepared material was immersed in a solution of ferric sulfate, whereupon it immediately turned deep blue on all exposed surfaces. Immediate sectioning of the piece showed a very thin layer of blue on all outside surfaces. This was replaced in the ferric sulfate solution, and after an hour was sectioned again. The blue ring had increased to a depth of approximately one-sixteenth of an inch. This procedure was repeated until it was found that the entire piece was affected throughout. It is unnecessary to estimate the time required inasmuch as this is undoubtedly a function of the size and configuration of the piece used. Temperature and concentration of the solutions also probably had some effect. However, this experiment was attempted only to see if salt solutions would diffuse into such an apparently dense substance as chilled agar in a 5 per cent concentration.

Potassium ferrocyanide is a very sensitive indicator for the presence of iron; therefore, the penetration of the iron solution into the agar was easily followed. Although one cannot say that salts would completely penetrate in all cases, this does indicate that the effect of metallic salts upon agar gels is more than a surface phenomenon.

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METHOD OF DETERMINING DIMENSIONAL CHANGE

A stainless steel master die was prepared, approximately one-half inch square by one and one-half inches, with slightly rounded corners to reduce cracking or "fining" of the agar gel upon removal of the master. This die was machined to dimension, the surface polished metallurgically, and finally measured to an accuracy of 0.0001 of an inch. It was then mounted on a stainless steel flat plate and surrounded by a removable matrix. This metal matrix was a solid ring (3½ in. I.D.) rather than perforated since the agar used contained no fillers and could not have been contained in a perforated ring as dental compositions are. A 5 per cent solution of agar in water was heated until solution occurred, cooled to 43° C. (110° F.), then poured over the master, care being taken not to entrap air bubbles. The assembly was allowed to cool in air for five minutes, then placed in room temperature water to the depth of agar within the ring (1½ in.) for a period of one-half hour. (Not submerged; the water did not come in contact with the agar gel.)

The amount of agar used for each specimen was kept constant by scoring the ring and pouring to that depth (1½ in.). This cooling technic is consistent with dental practice, although the time intervals are longer because of the greater bulk of hydrocolloid being used. After cooling, the die was carefully removed. The impression was subjected to various concentrations of potassium sulfate and other sulfate solutions to obtain the test data. They were allowed to remain in the respective solutions for prescribed lengths of time, after which the impression was removed from the solution and the cavity filled with a dental die stone* of known setting expansion, keeping spatulation and water/powder ratio constant. While the stone was setting, the impression was placed back under the solution at room temperature. After the stone had reached its final set, the agar was cut away from the specimen. After drying it was measured with a micrometer across the one and one-half inch dimension, averaging from ten to fifteen readings on each specimen. These measurements were compared with the original master, per cent change being recorded.

This measuring method was checked by the use of an optical micrometer comparator. The results differed only in the fifth decimal place, and would not give consistent checks beyond the fourth decimal place. In rounding off this fifth decimal place, the same results were obtained as with the micrometer, therefore it was decided that the micrometer was within reasonable accuracy, measuring in ten thousandths of an inch.

New agar† was used for each specimen. The specimens were run in triplicate for each point on the curves, and in cases where a smooth curve was not obtained, either the group of three was repeated, or time intervals close to such anomalous points were run. The agar was chosen from many raw materials because it gave a much cleaner solution, more nearly clear in solution, and was light in color. It was necessary to use a 5 per cent solution since higher con-

*Akratex, Whip-Mix Corporation, setting expansion, 0.05 per cent at 28 parts water to 100 of powder.

†Kobe No. 1, Strip agar-agar from the T. M. Duche Company of one shipment was used to eliminate error from possible difference in base material.

centrations gave gels which were very stiff and hard, presenting little elasticity and not resembling commercial brands of hydrocolloid. The 5 per cent solution did have the feel and texture of the commercial brands. The addition of fillers and other materials undoubtedly weaken the approximately 10 per cent solution as used in dentistry, which can be explained by the theory that foreign materials are trapped during micelle formation, separating the micellae, making a more elastic but less strong end product.

Other writers have found that gypsum base materials will not set in agar without the use of potassium sulfate or other accelerators. This was overcome by permitting the stone die to remain for twenty-four hours in the impression. The surface was then hard and glossy. It is only the surface that is retarded by the organic exudations from the agar; the bulk of the stone sets at the usual time; therefore, the dimensions should not have been affected.

It is realized that the metal die does not approach tooth structure or configuration; however, all errors from these factors will be common to all specimens; results are comparable within themselves. Dental impression materials are composed of 6 to 10 per cent of some type of agar, plus various fillers, salts, preservatives, coloring matter, flavoring agents, etc., any of which could make dimensional differences. Even different types of agars or agar from different sources might give different reactions in salt solutions. It would be desirable to use dental compositions in this same test method with proper modifications, such as the use of a perforated ring; but laboratory time was limited so that it was not possible for this paper.

DISCUSSION OF RESULTS

Fig. 1 shows the effect of exposure of the impression to air and of immersion in water for lengths of time between immediate pouring and an elapsed time of ten hours. For this test, the agar impression was set on an elevated, perforated aluminum plate to permit exposure to air on all sides.

Plotting the loss in dimension in per cent on the ordinate, and the elapsed time in hours as the abscissa, an almost geometric curve of gradual contraction is evident in the graph showing exposure to air. This is undoubtedly due to loss of moisture in the agar through evaporation in combination with natural syneresis. The curve shows a tapering off at ten hours, and a longer time interval probably would not show much more than a 2.0 per cent contraction within a measurable range. This was not carried through any greater length of time because the specimens were becoming quite distorted and difficult to measure with accuracy.

In the curve showing the effect of immersion in water, there is the same contraction during the first half-hour as is shown in the specimen exposed to air. For the period of the next half hour (total elapsed time, one hour) the agar had begun to expand, and the zero point was reached. The initial contraction was overcome. From this point, a gradual increase in expansion was noted until at four and one-quarter hours, a leveling off at an expansion of minus 0.266 per cent occurred. There was no further expansion beyond this

point. A specimen allowed to stand in water for twenty-four hours showed no increase or decrease in expansion over this maximum of 0.266 per cent. The greater length of time was not plotted on the graph. These results are roughly correlative with those found by Skinner[1] although he used commercial brands of hydrocolloids in his tests.

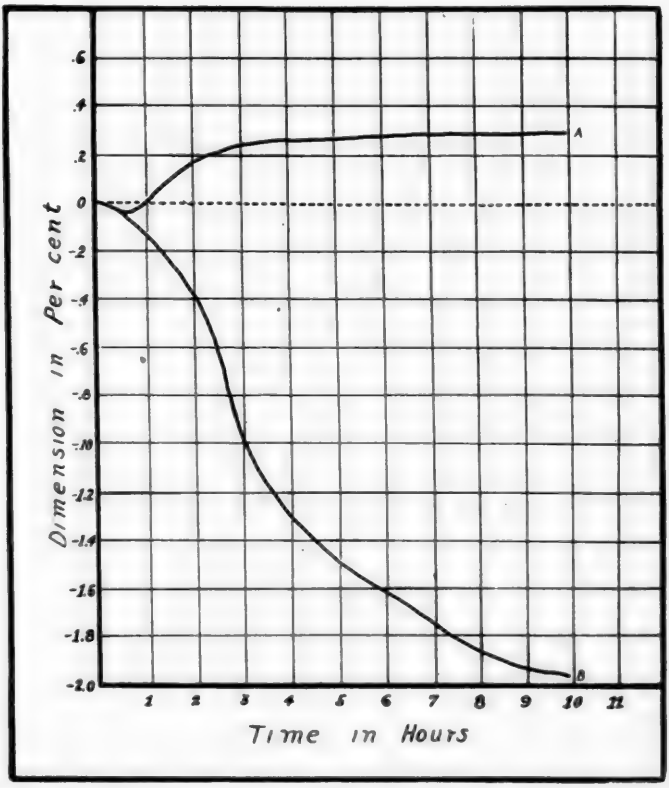

Fig. 1.—Effect of immersing 5 per cent agar in water (*A*), and effect of exposure to air (*B*).

Fig. 2 depicts the results of immersing the impression in a solution of 2 per cent potassium sulfate. There was a gradual contraction through a period of two hours, then the curve leveled off at a contraction of 0.51 per cent. This remained the same for three hours, then an expansion through another two-hour period, reaching a maximum of minus 0.25 per cent. This contraction of 0.25 per cent was constant throughout the rest of the time range, and a twenty-four hour check gave the same figures. From this curve, an agar gel reaches stabilization only after seven hours of immersion in 2 per cent potassium sulfate, and with a contraction of 0.25 per cent under the original master die size.

James[2] found that in using actual dies and castings, the inlays would not approach a seat if the impression (commercial brands of hydrocolloid) were left in water or 2 per cent potassium sulfate for a period of time longer than four hours. This may be due to distortion of the die as much as to absolute

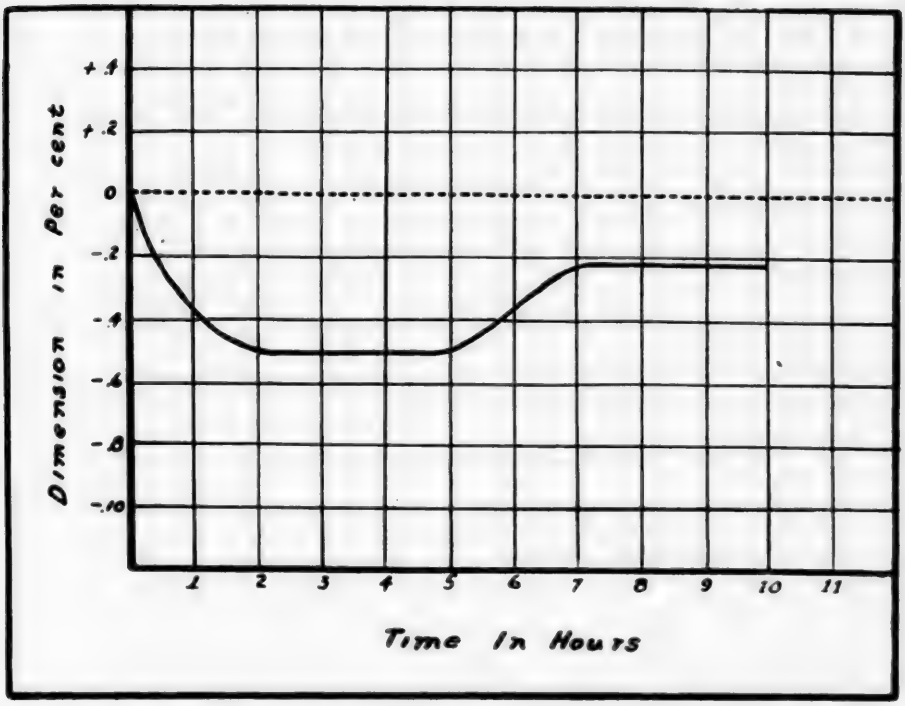

Fig. 2.—Effect of immersion in 2 per cent potassium sulfate.

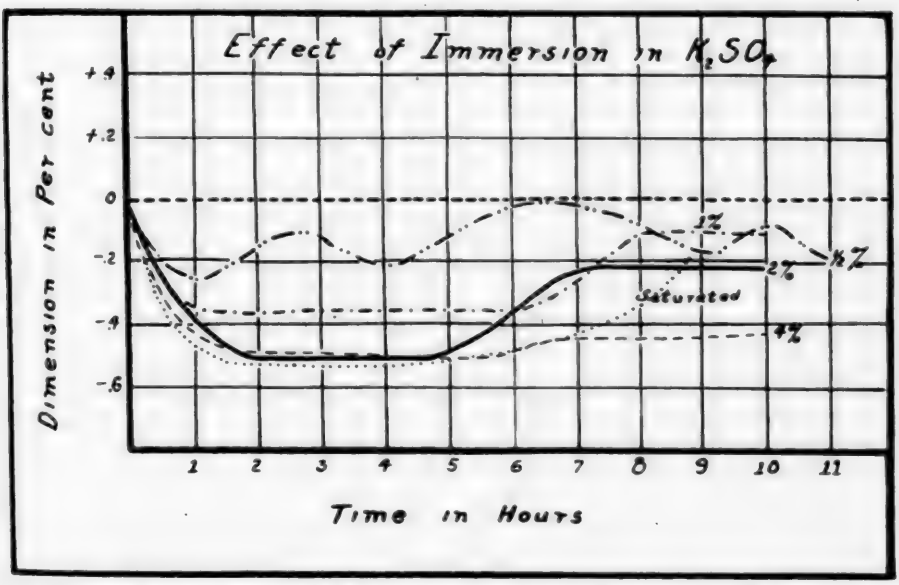

Fig. 3.—Comparative graph of various percentage solutions of potassium sulfate.

dimensional change in the material. Wherever there is a reversible change of dimension in materials in intricate shape, there is likely to be distortion even though the material should return to original volume.

It has been claimed[3] that a 2 per cent solution of potassium sulfate will stabilize agar gels because it is isotonic with the hydrocolloid composition. This may be true for a specific brand of hydrocolloid containing exactly 2 per cent of potassium sulfate as part of its formulation; however, it does not hold true for all commercial brands of hydrocolloid as shown by James,[2] nor is potassium sulfate a stabilizing agent for the agar base material as shown in this work. Even with a specific brand of hydrocolloid designed to be used with 2 per cent potassium sulfate, this stabilizing effect can be lost by dilution of the hydrocolloid with water as is the practice in many dental offices. This, of course, changes the original concentration of potassium sulfate in the material, and the concentration of the stabilizing solution would have to be changed accordingly.

Fig. 3 shows a comparison between various percentage solutions of potassium sulfate. The outstanding point of this group of curves is that regardless of the percentage of potassium sulfate in the solution, all of the curves follow a definite pattern of contraction-leveling off-expansion, and a final leveling off which is constant. The only exception is found in the one-half per cent solution, which is in a constant state of change. According to these results, a 1 per cent solution of potassium sulfate reaches a stabilization much nearer to the zero point (which is the original die dimension) than do any of the other concentrations, which suggested that a lower percentage solution might give better results. There is very little difference in the minimum points of all percentage solutions except the 1 per cent.

Fig. 4 shows the effect of three different metallic sulfate salts. Ferrous sulfate gave a curve that was in a constant state of change, although fairly constant up to an elapsed time of one hour. From that time through twelve hours, there was first an expansion to plus 0.14 per cent, then a constant contraction. However, these changes were very smooth and gradual without sharp increases and decreases in expansion, and without periods of leveling off of the curve. The sodium sulfate in a 2 per cent solution showed the typical pattern of the potassium sulfate, which was to be expected from the close chemical relationship between potassium and sodium. Although the same general pattern was followed by sodium sulfate, the minimum point was minus 0.32 per cent, whereas potassium sulfate in a 2 per cent concentration gave a minimal point of minus 0.51 per cent.

Nickel sulfate showed an enormous expansion during the first half-hour, a maximum of 0.34 per cent. The next half-hour showed a contraction to plus 0.03 per cent which was constant for about six and three-quarter hours. After this time, it decreased to exactly zero, which remained constant up to ten hours' time. Then there was a radical loss in dimension. In short, from a period of one hour to ten hours the measurements of the stone reproductions were very close to the original dimensions of the master.

In Fig. 5, aluminum sulfate shows the same high expansion as did nickel sulfate but over a period of two hours. At the end of this time, the impres-

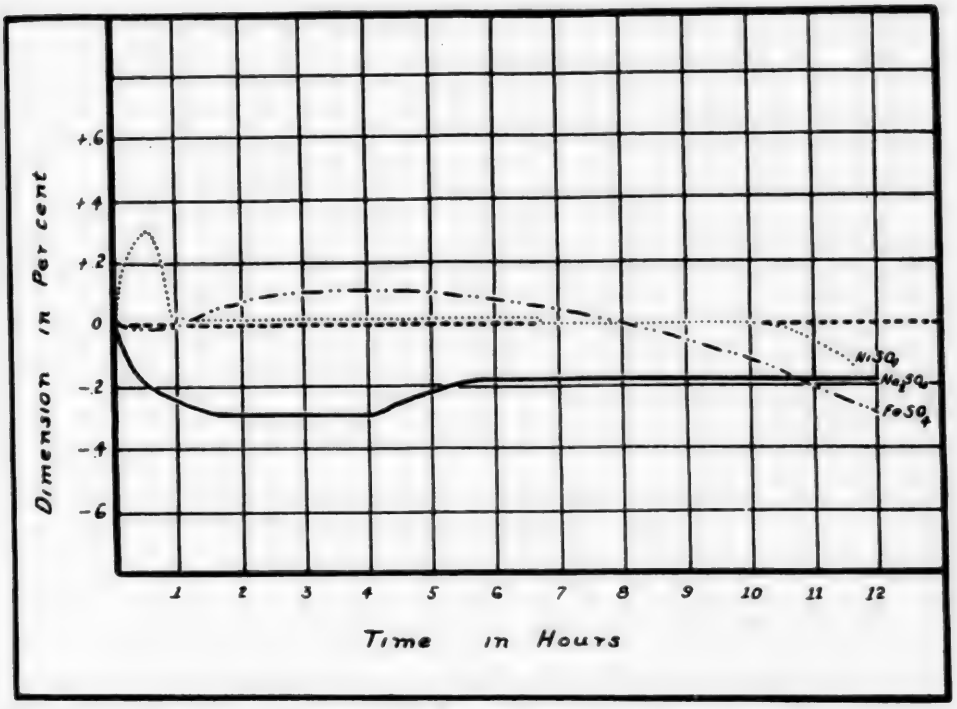

Fig. 4.—Effect of immersion in nickel, ferrous, and sodium sulfate.

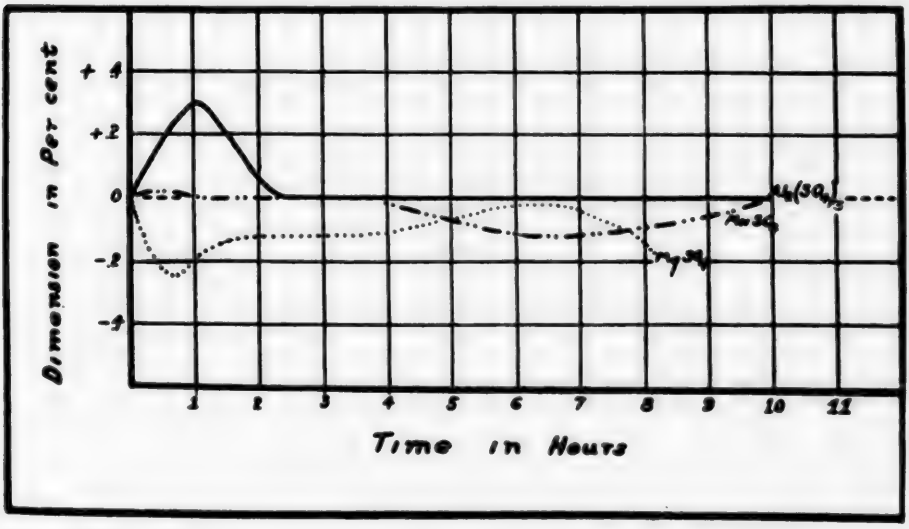

Fig. 5.—Effect of immersion in aluminum, manganous, and magnesium sulfate.

sion returned to its original dimensions, and remained constant throughout a twenty-four hour period. This suggests that aluminum sulfate would make a very good stabilizing agent for 5 per cent agar gel if the impression were permitted to stand longer than two and one-half hours before running the die. In this same illustration, magnesium sulfate shows no stabilizing effect. Manganous sulfate shows a stabilizing effect through a period of the first four hours of immersion, then a gradual decrease in dimension, and finally an increase back to the zero point.

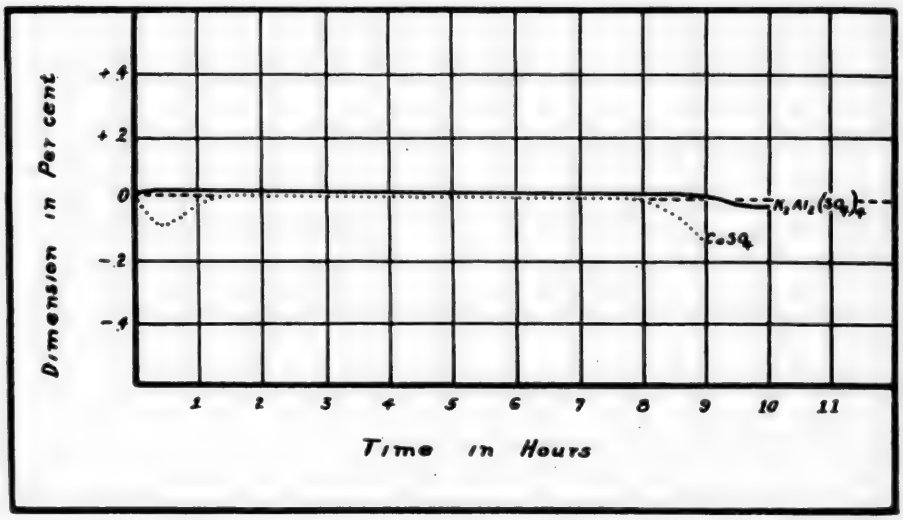

Fig. 6.—Effect of immersion in cobalt and aluminum-potassium sulfate.

Fig. 6 shows the effect of cobalt sulfate and potassium-aluminum sulfate. In the case of cobalt sulfate, the inverse of nickel and aluminum sulfate is observed in that there is a contraction during the first hour, then a leveling off at the die dimension through a period of eight hours. However, cobalt sulfate is undesirable as a stabilizing agent as it makes the impression extremely brittle and crumbly. These impressions had to be handled with great care, or they broke into many pieces during the running of the model. Potassium-aluminum sulfate showed the most consistent results of all the solutions tested. There is a very slight increase in dimension (plus 0.02 per cent) which is constant through a time range of nine hours. After this, there is a loss in dimension to a point of minus 0.03 per cent which is constant throughout a twenty-four hour period. Not only did potassium-aluminum sulfate show the best dimensional results, but the surfaces of the die material were much better—harder and smoother than in the case of many of the other salt solutions.

CONCLUSION

Results here show that potassium sulfate does not hold an agar gel to the original measurement through any period of time. In all cases there was a

definite contraction, the greatest occurring during the immersion period of two hours to five hours. The maximum contraction was found to be minus 0.51 per cent in most concentrations of potassium sulfate. The minimum was found to occur after five hours of immersion in a 1 per cent solution, at minus 0.37 per cent. The gel is so unstable in a one-half per cent concentration that it cannot be considered.

Of the other salt solutions tried in this work, the best are aluminum sulfate, cobalt sulfate, nickel sulfate, and aluminum-potassium sulfate. All of these follow the line of the original master within reasonable limits. However, all of these salts except potassium-aluminum sulfate showed either a great amount of expansion or contraction during the first two hours of immersion. The double salt of potassium-aluminum sulfate showed no great deviation from the original die dimension during the entire time of immersion.

These results must be considered with caution, inasmuch as they probably are not directly transferable to dental applications per se. Dental compositions are composed of many things other than agar and water; any or all of these additives may make a considerable difference. Also, it must be remembered that the form chosen for these measurements did not simulate tooth structure. In this case, the penetration of the salt solutions was more uniform because a convoluted surface was not presented to the solutions; possibly less distortion was encountered in this work than would be the case with a normally intricate inlay die.

It is possible that in the smaller bulk surrounding an actual impression of the mouth the contraction would be minimized. Also, there is the question of just how far off a die may be on a practical case without there being a noticeable misfit to the average dentist. There has been no published work on the tolerance permissible between a casting that is too large and one that is too small. Therefore, there is no one who can say how much dimensional change can be tolerated in the impression material, although it stands to reason that the closer the impression material stays to the original dimensions of the die, the less distortion is likely to be encountered, and the more accurate the reproduction.

It may be that each brand of reversible hydrocolloid impression material requires its own solution for stabilization against dimensional change, supplied by the individual manufacturer according to the formulation. This work suggests the probability of finding such solutions.

This work was done at the University of Louisville under the direction of Dr. Max I. Bowman, Professor of Chemistry. Part of the work was presented as a thesis in fulfillment of the requirements for an A.B. degree, and the remainder carried on as independent research, also under Dr. Bowman. The materials, die, etc., were furnished by the Whip-Mix Corporation, whose cooperation is greatly appreciated.

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A COMPARISON OF THE TENSILE AND BENDING PROPERTIES OF DENTAL GOLD WIRES

D. F. TAYLOR, M.S.E.,* AND F. A. PEYTON, D.Sc.

School of Dentistry, University of Michigan, Ann Arbor, Mich.

INTRODUCTION

MODERN dental practice makes wide use of precious metal alloys in wire form as partial denture clasps and as orthodontic appliances. When used in such applications, these alloys are subject only to bend loading and never to pure tensile loading. For this reason, there has long been a feeling within the dental profession that the tensile test for wires was somewhat inappropriate and might be replaced advantageously by a bend test. However, the absence of any previous correlation between the test values obtained from the two types of loading, as well as the large amount of effort represented by collected tensile data on these alloys, has heretofore prevented any serious consideration of a possible change of standards from one test to the other. The technical representatives of several manufacturers have displayed a continuing interest in the development of possible relationships between tension and bend tests. This interest was expressed through the loan, by these manufacturers, of gold alloy wires of high intrinsic value for use in this investigation. Further support was received from the Office of Naval Research which sponsored the project as a part of its program of research on the properties of restorative dental materials.

The specific purpose of this investigation was to determine what correlation, if any, existed between the mechanical properties of wrought gold alloys as found in a standard tensile test and those as found in a bend test. The type of bend test used in this investigation was the cantilever loading of a wrought gold wire. It is believed that the conditions of such a bend test much more nearly approach the actual conditions under which these alloys are used than does the tensile test.

The program of the project was set up as follows: An attempt was made to obtain a wide range of inherent mechanical properties in the alloys to be tested, at the same time reducing as far as possible other variables such as wire size, sample length, and heat treatment. A total of twelve alloys was selected, all of the same size and all to be subjected to identical heat treatment and testing. These alloys were to be tested in two conditions, fully soft (10 min. at 700° C., quench), and age hardened (softened as above, then 15 min. at 360° C.). It was recognized that this hardening practice might not produce either the maximum or optimum properties of any one alloy, but it was

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*Present address: Washington University School of Dentistry, St. Louis, Mo.

employed to eliminate possible variation resulting from differing heat treatments, and because it could be controlled more accurately than oven cooling. For each alloy in each condition three tensile tests and three bend tests were planned. It was proposed that stress-strain curves be taken for each specimen and the properties of the wires calculated from the curves. The desired comparison of the two tests was to be obtained from the average values of the three specimens of each type. This method was followed as closely as possible and in general the results obtained were quite satisfactory.

A separate investigation is being made of the microstructures of these alloys and will be reported separately.* In this study no immediate attempt was made to correlate the results of the mechanical tests with the microstructure of the alloys. The equilibrium relationships for the alloys used are complex, since all of the alloys have five or more components present to the extent of 0.5 per cent or more, and some alloys may contain as many as seven major components and traces of others. Considering the complexity of the dental gold alloy system, only a relatively small amount of study has been devoted to the investigation of its properties. The binary systems are known[1] and some work has been done on ternaries, but of the more complex systems only portions of two pseudobinaries, in the platinum-gold-silver-copper and palladium-gold-silver-copper quartenary systems are available.[2, 3]

METHOD OF INVESTIGATION

Three alloys from each of four well-known manufacturers were selected for the test. The selection was made on the basis of data available in manufacturers' physical property charts. An attempt was made to select alloys that would give a fairly even distribution of values over the entire range of tensile properties and at the same time to select one inherently soft, medium, and hard alloy from each manufacturer. The nominal chemical compositions of the alloys selected are given in Table I.

These alloys were received as 1-foot lengths of 0.040 in. wire, which were cut into 6-inch lengths for heat treatment. A bundle of 6-inch wires was bound together with stainless steel ligature wire and suspended in a vertical muffle

TABLE I

NOMINAL COMPOSITIONS

ALLOY	WEIGHT PERCENTAGE						
	AU	PT	PD	AG	CU	ZN	NI
1	56.5	---	5.0	25.0	11.0	---	2.5
2	66.6	4.1	---	12.5	15.8	1.0	---
3	61.5	2.5	3.0	14.5	18.5	---	---
4	63.0	2.0	2.0	14.5	18.0	---	0.5
5	30.0	5.1	10.1	37.6	17.2	---	---
6	63.0	7.0	5.0	10.5	12.0	0.5	2.0
7	60.0	16.0	---	12.5	9.6	1.2	---
8	63.0	7.0	5.25	10.0	12.25	0.5	2.0
9	55.0	17.0	7.5	8.0	11.0	1.5	---
10	54.3	17.0	7.0	8.2	12.5	1.0	---
11	55.0	17.0	7.0	6.5	13.0	1.5	---
12	15.0	9.0	37.0	21.0	15.0	1.0	2.0

*J. Pros. Dent. 1: 177, 1951.

electric resistance furnace. A chromel-Alumel thermocouple was inserted into the middle of the bundle, and a Leeds and Northrup semi-precision potentiometer was used to measure the temperature of the wires.

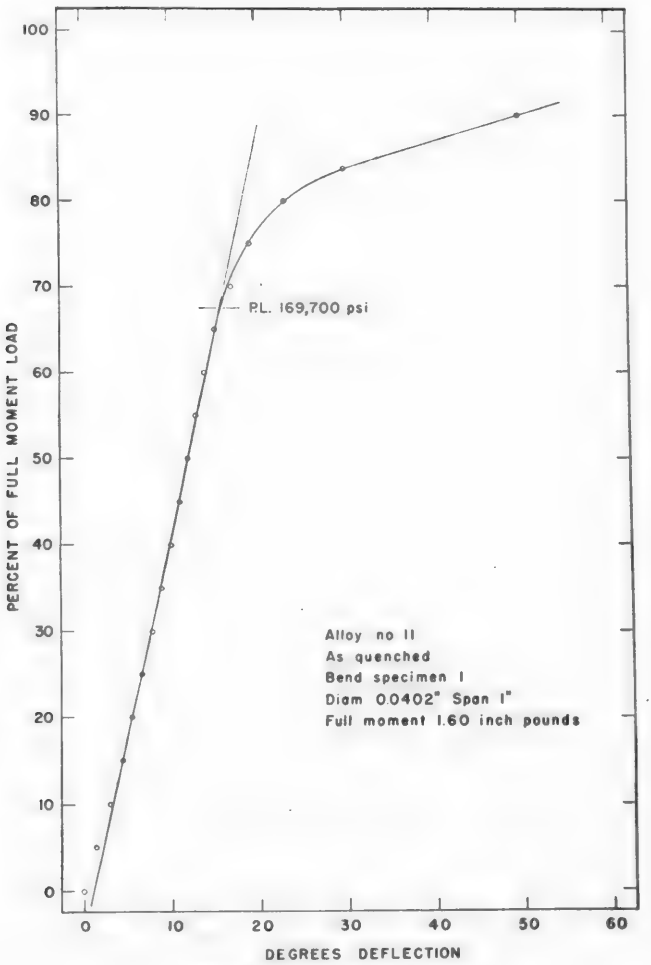

Fig. 1.—Typical bend curve.

The heat treatment used was as follows: The bundle of wires was inserted into a furnace preheated to 700° C., and held there for 10 minutes, reaching furnace temperature within 2 to 3 minutes. After 10 minutes in the furnace, the bundle of wires was quenched in room temperature water. This treatment corresponds to the American Dental Association specification No. 7 recommended softening treatment. For those specimens to be tested in the hardened condition, this softening treatment was followed by aging in a salt bath for 15 minutes at 360° C. and water quenching to room temperature.

Three wires from each bundle were selected for tensile testing and one for bend testing. The wire to be used in the bend test was cut into three 2-inch lengths which were sufficient for that purpose. The instrument used was a Tinius Olsen stiffness tester of five inch-pounds' bending moment capacity, similar to the instrument described by Shell.[4] For this investigation, the moment arm was fixed at 1 inch while the weights were varied to fit the anticipated properties of the materials. Load increments of 5 per cent of the full moment load were used, with additional points being taken after yielding started.

The tensile specimens were tested in a Tinius Olsen tensile testing machine of 1,000 pounds' total capacity. It was of the mechanical drive type, equipped with an auxiliary motor that gave a head travel of 0.024 inch per minute. Loading was stopped while readings were taken; the resulting effective loading rate was approximately 20,000 pounds per square inch (psi) per minute. Readings were taken at 5-pound intervals and the strain was read to the nearest 0.0001 inch. The gauges used were of the type developed at the National Bureau of Standards for this purpose.[5] They are made in matched pairs mounting on opposite sides of the wire. They thus avoid eccentrically loading the wire because of uneven distribution of gauge weight. This mounting also compensates for small bends left in the wire by previous treatments, the average of the two dials being a good average strain for the whole cross section. To minimize further the effect of crooked wires, initial readings were taken at a 5-pound load.

Using this method, and with assistance in reading the second tensile gauge, three bend and three tensile specimens were tested for each alloy in both the hard and soft condition. Two additional series of tests were made in the soft condition with the result that twenty-six average tensile values and corresponding bend values were obtained.

EXPERIMENTAL RESULTS

In Figs. 1 and 2 are plotted the typical results for bend and tensile tests. Both curves represent the data for Alloy 11. Results from other alloys give comparable curves. These curves were plotted in terms of the measured units (i.e., load and strain or deflection), and the stress values were calculated after the proportional limit and modulus were determined on the graph of each specimen. The proportional limit was taken as that point where the best curve through the points visibly deviated from a straight line. This differs slightly from the value normally reported for these alloys,[6] which is determined as the point at which the slope of the curve is 1.25 times the elastic slope. The absolute difference is in most cases very small, as most of the alloys tested tend to elongate very rapidly once the proportional limit is reached. The method used in this project was selected because it was independent of the plastic strain rate, and because the formulae used for calculating stresses in the bend tests apply only within the truly elastic range of the material. Tables II and III report a summary of the experimental data on proportional limit, ultimate tensile strength, and modulus of elasticity.

TABLE II

MECHANICAL PROPERTIES

ALLOY	PROPORTIONAL LIMITS		ULTIMATE TENSILE STRENGTHS
	BENDING EXPERIMENTAL PSI	TENSION EXPERIMENTAL PSI	EXPERIMENTAL PSI
1	83,600	51,700	90,700
1a	76,500	51,500	92,300
2	71,700	49,450	75,200
3	78,600	49,700	81,200
4	83,500	55,000	86,700
5	97,100	58,000	87,500
6	127,000	67,000	107,300
6a	119,600	69,200	110,500
7	113,900	65,700	100,800
8	129,700	87,000	115,800
9	122,900	66,900	108,200
10	145,400	72,400	114,800
11	167,500	95,250	130,800
12	129,800	79,800	126,000
1h*	189,500	97,800	140,500
2h	146,300	84,870	112,700
3h	168,200	92,500	129,200
4h	169,530	101,430	130,700
5h	140,000	89,300	115,200
6h	211,100	129,370	163,800
7h	162,700	94,800	128,500
8h	198,530	128,230	152,800
9h	162,200	100,730	135,700
10h	178,130	107,700	135,700
11h	215,400	129,600	167,800
12h	174,300	107,830	142,200
	Independent Data		
A	95,000	61,000	
B	113,000	67,500	
C	94,700	53,000	
D	84,800	60,300	
E	130,000	74,000	

*h denotes hardened condition.

In Fig. 3 is plotted the experimentally determined proportional limit in bending versus the log of the proportional limit in tension, as reported in Table II. Also shown are five points as determined independently by another investigator[7] on wires of similar size and composition. It will be noted that, on this type of plot, the data can be represented by a straight line. The equation of this line, excluding the independent points, was found by the method of least squares to be $\text{Log } P_T = 3.004 \times 10^{-6} P_B + 4.478$.

An attempt was made to account for the deviation of various points from this line. It was found to be impossible to do so, however, with respect to any of the variables upon which data were available. The variable which might be expected to have the most effect, wire diameter, was kept within quite narrow limits. The nominal size of all of the wires used was 0.040 inch; the actual diameter of the specimens after heat treatment ranged from 0.0396 to 0.0405 inch. Over this range the effect of size, if any, was concealed by individual variations between alloys.

TABLE III

MODULUS OF ELASTICITY

ALLOY	BENDING EXPERIMENTAL 10^6 PSI	TENSION EXPERIMENTAL 10^6 PSI
1	12.523	12.050
1a	12.250	11.330
2	11.102	11.172
3	11.883	12.217
4	12.187	11.020
5	11.990	11.030
6	14.300	13.330
6a	13.530	12.970
7	13.273	11.990
8	15.157	12.967
9	13.880	14.420
10	14.817	14.283
11	16.217	14.490
12	17.097	18.017
1h	14.950	13.073
2h	13.513	11.703
3h	14.910	13.090
4h	14.473	12.210
5h	13.160	13.300
6h	17.127	14.327
7h	14.663	12.883
8h	16.393	13.699
9h	16.030	14.833
10h	18.013	15.173
11h	17.483	15.157
12h	19.217	17.867

The values obtained for modulus in bending and in tension are plotted against each other in Fig. 4. This plot exhibits much more scatter than appeared in that of the proportional limits. While the general relationship between the two tests is fairly apparent, it is confused by the deviation of individual alloys from the mean. As a mechanism to determine this trend somewhat more accurately, a plot was made (Fig. 5) of the change of modulus in tension versus the change of modulus in bending for each alloy between the soft and hardened conditions (see Table IV). From this figure it can be seen that, in general, the effect of any aging heat treatment is to increase the modulus both in bending and in tension. The increase of the bending values is greater than that of the tensile values. The only two points that violate these general rules are those of the two alloys that do not fall within the normal composition range for these alloys. One alloy (alloy 5) has silver for its main component, and the other (alloy 12) has palladium as a base (see Table I). Modulus values as determined in this investigation seem to be more subject to the influence of variables which could not be controlled than are the values obtained for proportional limit. The method by which they were determined, drawing by eye the best straight line through the plotted points, is admittedly subject to error. The adoption of a more precise method, a differential curve perhaps, did not seem justified in what was essentially an extensive rather than an intensive investigation.

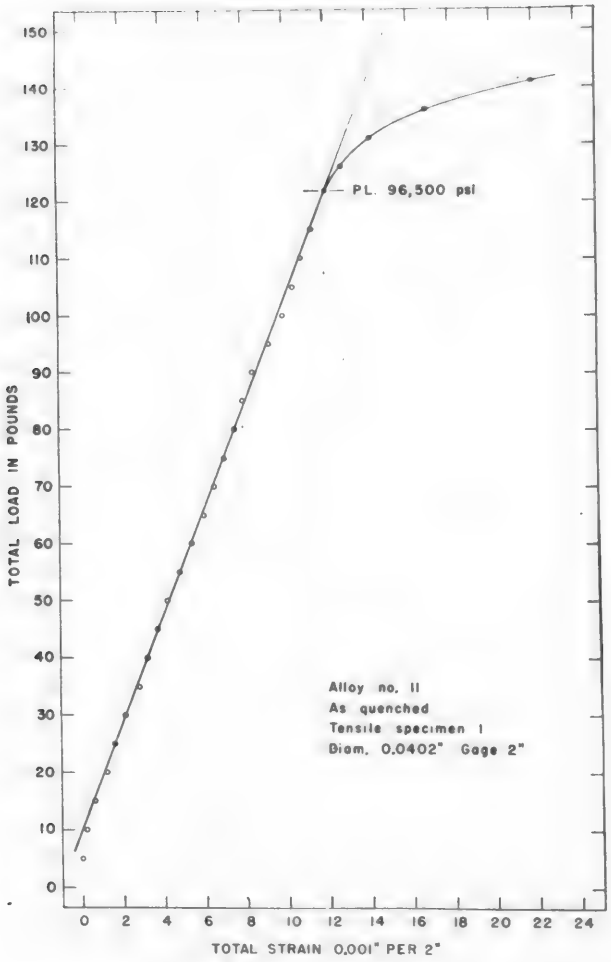

Fig. 2.—Typical tensile curve.

TABLE IV

CHANGE IN MODULUS ON HEAT TREATMENT

ALLOY	MODULUS TENSION	MODULUS BENDING
1	1,023,000	2,420,000
1a	1,743,000	2,700,000
2	531,000	2,411,000
3	873,000	3,027,000
4	1,180,000	2,286,000
5	2,270,000	1,170,000
6	997,000	2,827,000
6a	1,357,000	3,597,000
7	893,000	1,390,000
8	732,000	1,240,000
9	463,000	2,150,000
10	890,000	3,196,000
11	661,000	1,266,000
12	−150,000	2,120,000

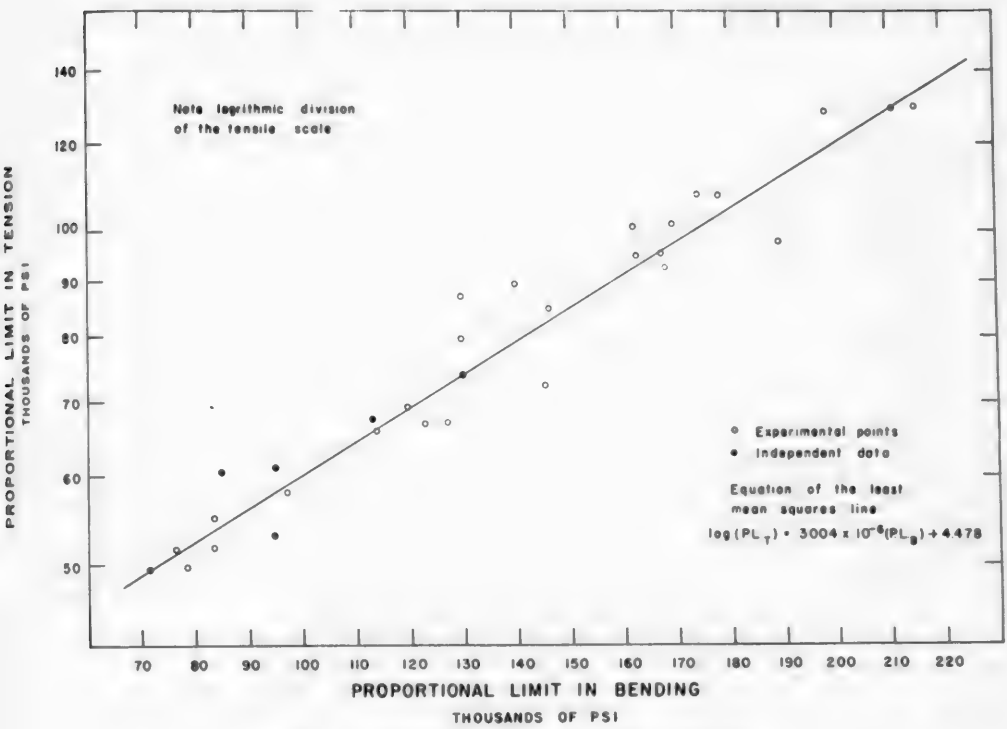

Fig. 3.—Proportional limits, bending vs. tension.

The method used seems to have been in general well suited to the scope and purpose of the project. The limited number of specimens tested for each condition precluded any weighing or statistical treatment of the data, yet for the proportional limit data the average probable error for the individual points is 2.58 per cent in tension and 2.18 per cent in bending. The probable error for an individual reading with respect to the least mean squares curve is only 4.76 per cent for the experimental data taken. If Alloys A to E from Table II are included, the probable error is 4.65 per cent.

The equipment used was checked for accuracy as far as possible. The stiffness tester consists essentially of two moment arms and a set of weights. These were measured and found to be correct. The tensile machine was checked against a standard test ring and compared with another machine of the University of Michigan Engineering Mechanics Department, which was known to be accurate. As a final check, two unidentified specimens of each of three alloys were sent to another laboratory for tensile tests. The results show good agreement with the values obtained in this investigation. These results are compared in Table V.

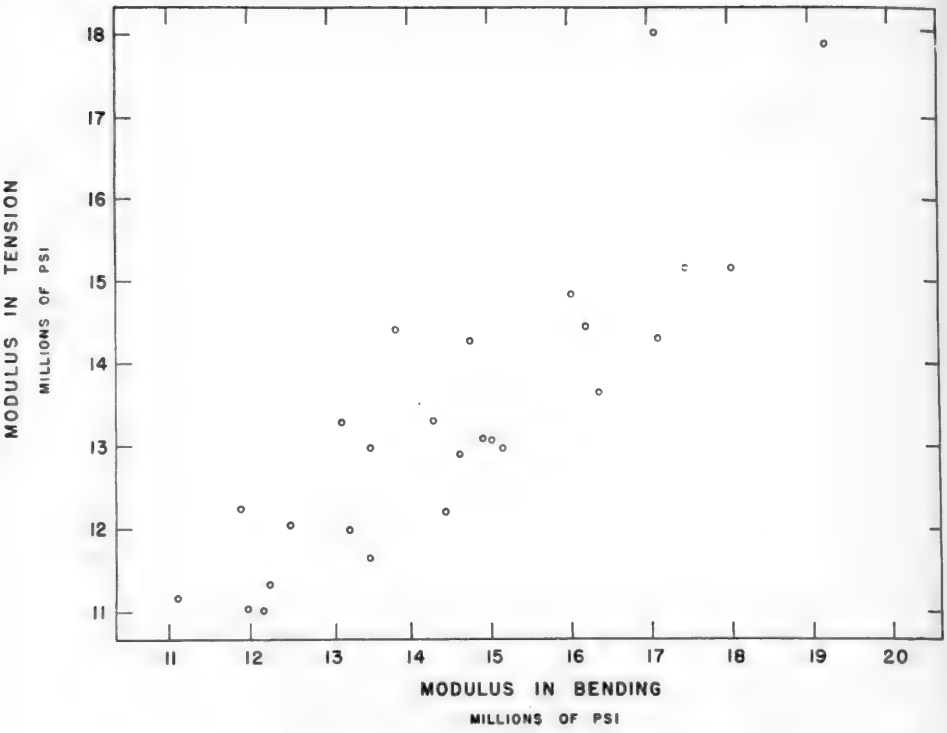

Fig. 4.—Moduli of elasticity, bending vs. tension.

TABLE V

CHECK ON TENSILE RESULTS

ALLOY	ULTIMATE TENSILE STRENGTH		PROPORTIONAL LIMIT	
	TEST VALUES	INDEPENDENT LABORATORY VALUES	TEST VALUES	INDEPENDENT LABORATORY VALUES
8h1	152,800	158,000	128,230	126,500
8h2	152,800	158,000	128,230	126,500
9h1	135,700	135,000	100,730	90,000
9h2	135,700	135,000	100,730	93,800
10h1	135,700	136,500	107,700	101,000
10h2	135,700	133,000	107,700	101,000

ANALYSIS OF RESULTS

Every method of physical testing to determine the properties of a material involves some inherent assumptions. In the case of the tensile test, the primary assumptions are few and deviate so little from actuality for most materials that they introduce very little error in the calculated properties. The two main assumptions are that the area of the cross section remains constant. Within the elastic range these assumptions are reasonably exact, and the only property involving them that is taken from the plastic range is the ultimate tensile strength. This value is always calculated on the original area for design purposes, and

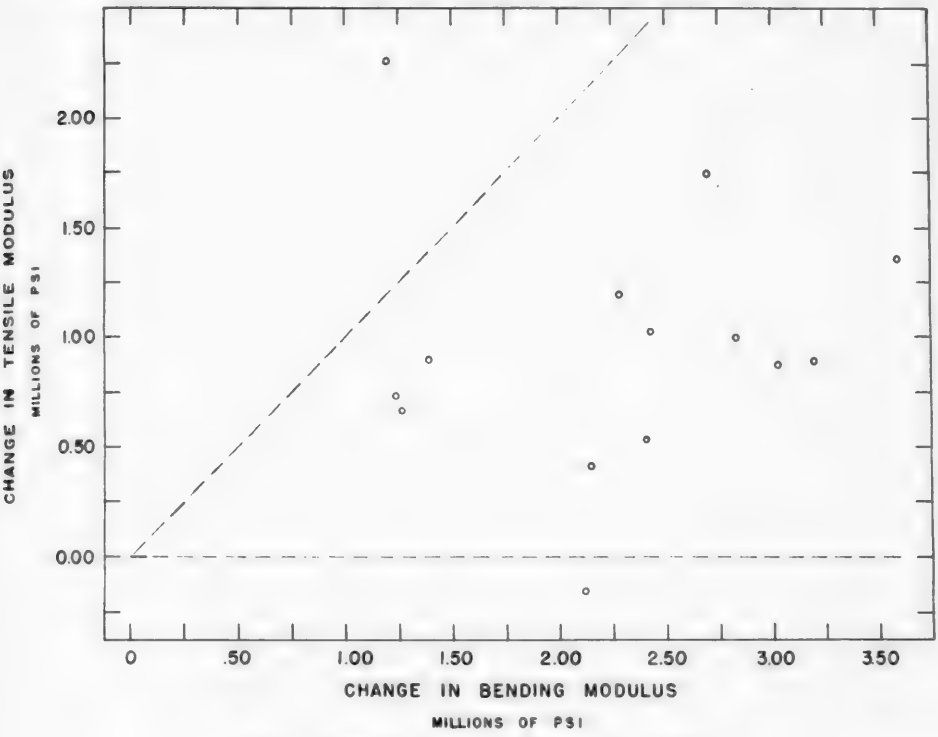

Fig. 5.—Change in moduli, bending vs. tension.

actually represents the stress at which the cross-sectional area is decreasing faster than the material is work hardening, rather than the maximum stress the material will bear.

The assumptions involved in a bend test are more complex and tend to introduce a larger deviation from the true properties of the material than those in the tensile test. In calculating the properties in a cantilever bend test, it is assumed that the behavior of the material is truly elastic up to the observed proportional limit, that the neutral axis of the wire does not shift during loading, and that the material has identical properties in tension and compression. It is also assumed that the stresses, deformation, and cold work introduced by the clamping process can be neglected.

Current theory states that if sufficient data are available to calculate the errors introduced by the failure of the above assumptions to hold true, the properties of a material in bending and in tension will be found to be equal. The fact that the values obtained from using the two tests are not the same indicates that some or all of the assumptions made for the bend specimens are inexact. This, however, does not of itself invalidate the bend test, but rather supports it, in that it demonstrates the inapplicability of data derived in one situation for use in the other.

It is at present impossible to design a dental appliance rigorously from tensile data. To do so would require unavailable data on the compressive properties, the shift of the neutral axis, and the effect of end conditions for dental alloys subjected to bending. However, it would be possible to design an appliance from data derived from a bend test. The total effect of all the undetermined deviations from ideality has been included in the data by using a test situation similar to the desired application.

In practice, dental appliances are never designed from the basic mechanical data. The properties reported in physical property charts are used mainly as a basis for the comparison and selection of alloys. For this purpose, bend test data are equally as satisfactory and offer the added advantage of having been derived from a situation as nearly the same as the intended use as is possible.

In selecting the alloys for use in this investigation, no attempt was made to choose them on the basis of composition. As a result, a wide range of composition is represented in the alloys tested. Similarly, during testing no special effort was made to insure uniform clamping conditions. Yet, in spite of these two factors, the random variation of which would be expected to increase the scatter in the data, a close correlation was obtained between the two test methods (Fig. 3). From this it can be anticipated that using uniform clamping conditions for the bend test will result in an improved correlation between the two tests, and that a single curve will suffice for the entire composition range of wrought dental gold alloys.

SUMMARY

An investigation was made of the relative values of the mechanical properties of precious metal wires in tension and in bending. The properties determined were modulus of elasticity and proportional limit in bending, and modulus of elasticity, proportional limit, and ultimate strength in tension.

A series of alloys in the form of 0.040 inch diameter wires was tested by means of a standard tensile test, and by a cantilever loading bend test. Each alloy was tested, first in the solution treated (soft), and second in the solution treated and aged (hard) conditions. The final values recorded for each alloy condition were the arithmetic averages of the data from three tensile specimens and three bend specimens. To obtain these individual values, stress-strain curves were plotted for each specimen, and the apparent proportional limit and modulus were determined from each curve.

The values found for proportional limit ranged from 50,000 psi to 130,000 psi in tension and from 70,000 psi to 215,000 psi in bending. A definite correlation between the results of the two tests was found. When plotted on semi-log paper it yields a straight line having an empirical equation of the form $\text{Log}_{10}P_T - aP_B + b$ (1) where a and b are constants, P_T is the proportional limit in tension and P_B is the proportional limit in bending. a was found to be 3.004×10^{-6}, and b was found to be 4.478.

Earlier data by another investigator for similar alloys on specimens of the same size fit this curve very well. The average probable error of conversion from bend values to tensile values for the data of this investigation is 4.76 per cent; including the independent points it is 4.65 per cent. The alloys tested (covering the normal range of composition for wrought dental alloys) fit this single correlation in spite of varying clamping conditions in the bend test. The use of standard clamping conditions can be expected to yield an improved correlation unaffected by normal differences in alloy composition.

The results for modulus were more widely scattered but certain trends can be noted. A wide range of values was found for modulus in both tests. Tensile values ranged from 11×10^6 psi to 18×10^6 psi, while bend values ranged from 11×10^6 psi to 19×10^6 psi. As far as is known, this is a greater range than has previously been reported.

For most alloys and heat treatments, the modulus in bending is greater than that in tension. The effect of aging was to increase the modulus, both in bending and in tension, as the proportional limit was raised. An aging heat treatment increased the modulus, as found in bending, more than that found in tension.

Work is being continued on this project with the intention of extending it to a greater scope. It is believed that this comparison of tensile and bend properties of wires may be valuable, not only to the dental profession, but the correlation between tension and bending properties may be useful to those in industry where wires are employed.

The authors wish to acknowledge the cooperation of the Handy and Harman (Dee) Company, the J. M. Ney Company, the Spyco Smelting & Refining Co., and The S. S. White Dental Mfg. Co., and their assistance in providing wire for this study.

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ERRATUM

The article by Koser and Fisher, "Vitamin Requirements of Oral Lactobacilli," *J. D. Res.* **29**: 760-773, 1950, contains a table in which broken type resulted in erroneous data. In Table III, p. 768, the last column (Thiamine) should have plus signs opposite Cultures 78, 1002, 1013, and 1015, in addition to those which are clear.

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the Pacific Northwest Section

portland, oregon—oct. 22, 1950

Compiled by b. o. a. thomas, *Editor, Pacific Northwest Section*

1. Apparent Mechanism of Action of Sodium Azide as a Selective Inhibitor of the Genus Candida (Monilia). *Marie S. Slawson and Marshall L. Snyder, Department of Bacteriology, University of Oregon Dental School, Portland, Ore.* No difficulty was encountered in confirmation of Diamond's claim that sodium azide in a 1:10,000 concentration in tomato juice peptone agar (pH 5.0) selectively inhibited the genus Candida. In our hands it proved so effective in prevention of overgrowths by these organisms that it was included as an essential ingredient in this medium which is routinely used for lactobacillus counts. The only exception of importance was the relatively rapid loss of fungistasis in plates of tomato juice peptone agar held in the refrigerator. This limit was established to be no more than five days for media stored in this form. Fungistasis was retained indefinitely in medium sealed off from the atmosphere. Comparison of effectiveness of sodium azide on other members of the oral flora indicated that the mechanism of this selective action of sodium azide on the genus Candida was by interference with cytochrome-cytochrome oxidase systems of these strictly aerobic organisms.

2. Formation of Mucinous Polysaccharides From Sucrose by Oral Bacteria. *Doris G. Osborne, Howard M. Hackedorn, and Marshall L. Snyder, Departments of Biochemistry and Bacteriology, University of Oregon Dental School, Portland, Ore.* Preliminary work was carried out with the S20B strain of *Streptococcus salivarius* to establish procedures for isolation and analysis of polysaccarides formed from sucrose. Amounts of levans were markedly increased by twice daily neutralization of the 5 per cent sucrose broth cultures. Maximum yields of this polysaccaride were thus obtained within 48 hours. About half the fructose contained in the sucrose was converted to the levan. The balance was probably utilized for the energy requirements of the culture. Oral strains of streptococci which produced different mucinous colony types on sucrose agar were studied in respect to levans. Fifty strains obtained from 30 children were surveyed for this synthesis. All but one of these strains produced levan in significant amounts.

3. Natural Radioactivity of Human Teeth. *Jack De Ment, University of Oregon Dental School, Portland, Ore.* The natural radioactivity of human teeth is largely due to the 0.012 per cent of beta and gamma emitting $_{19}K^{40}$ present in all natural potassium; traces of uranium, radium, thorium, and radiocarbon account for a small fraction of the activity. An order-of-magnitude upper limit of the radiopotassium content of teeth, based on the highest reported assay value, can be estimated as roughly 10^{-4} per cent. Autoradiographs of adult molars, transversely sectioned just above the coronal line, were obtained with an Eastman NTB, 50 micron nuclear track plate, the exposure being 2065 hr. Controls were used to eliminate images due to pressure, long-period phosphorescence, and the Russell effect. Differences in potassium distribution appear between the enamel, dentin, root canal, and carious areas in both enamel and dentin. Carious areas show a much higher relative radioactivity than other regions of the tissue. Areas of high relative activity also appear at the dentino-enamel margin and the enamel surface. This work also indicates that tooth potassium may be present in an insoluble form.

4. Further Studies on the Anodic Decalcification of Teeth. *E. B. Jump and Jack De Ment, University of Oregon Dental School, Portland, Ore.* Using an apparatus previously described (*Science* 112:438, 1950), the authors studied a number of electrolytes varying in pH from 2.2 to 8+. Fragments of enamel matrix of adult human teeth were recovered following decalcification in 2 per cent NaCl, 90 per cent ethanol-2 per cent nitric acid, and dilute solutions of thioglycollic acid. Antioxidants in the electrolytic bath seemed to increase the amount of matrix recovered. It is apparent that "demineralization" is a gradual, continuous process. Specimens left in the bath an excessive time were completely destroyed—including all dentinal and enamel matrix; those treated for a shorter period exhibited a soft amorphous film which, when smeared on a slide and stained, presented the morphology of enamel. Some of this material when dehydrated and embedded for sectioning had recovered its hardness and could not be cut with the microtome. Enamel treated by these methods appears to be composed of masses of fibrillae arranged longitudinally in the characteristic prismatic form. These exploratory studies raise questions of the validity of assumptions about the composition of enamel based on staining reactions and whether it is possible to obtain a completely "decalcified" matrix from enamel.

5. Cooling Curves of Inlay Rings. *H. L. Gaskill, M.S., Ch.E.* The question is frequently asked, "Does the elapsed time in removal of casting ring from burn-out furnace until casting is done materially affect the dimension of the finished casting?" Thermal contraction of investment data shows that a 10° F. temperature variation is allowable to maintain 0.01 per cent dimensional accuracy. The cooling curves of inlay rings under various conditions were determined. From the data obtained it is apparent that to maintain this accuracy a variation of only three seconds in total elapsed time is allowable when the ring is removed from the oven at 1,300° F. With the ring initially at 950° F., seven seconds variation in total elapsed time is allowable.

6. Partial Results of a Clinical and Questionnaire Dental Survey of 6,010 Elementary Grade School Children of Seattle, Washington. *Frank T. Bowler, University of Washington, Seattle, Wash.* 6,010 school children between the ages of 5 and 10 years, chosen as a random sample from a possible 60,000, were examined by dentists using mouth mirror and explorer in good light. Attention was given to D.M.F. teeth, both primary and permanent, and also to existing oral hygene. Previous to this examination a questionnaire was filled out containing 32 questions about toothbrushing habits and candy, gum, and soft drink habits, worded to produce as uninfluenced answers as possible. It was found that the percentage of children with one or more D.M.F. permanent teeth ranged from 11.7 per cent in the 5-year group to 99.2 per cent in the 18-year group. Fifty-seven per cent of all the ones who had been to a dentist had never had x-ray films made. The per cent of children brushing after meals ranged from 59.3 per cent after breakfast to 7.7 per cent after supper in the 12- to 13-year group to 52.4 per cent after breakfast to 18.2 after supper in the 5- to 6-year group. The trend of better oral hygiene found clinically compared in an increasingly favorable ratio with the children's positive answers to the questions—"How many times a day do you brush your teeth?" and "How satisfied are you with the way your teeth look now?"

INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Scientific Proceedings of the New York Section
College of Dentistry, New York University—Nov. 9, 1950*

Compiled by Frances Krasnow, *Editor, New York Section*

1. Radioiodine Penetration Through Intact Enamel, With Uptake by Bloodstream and Thyroid Gland. *Herbert J. Bartelstone, V. A., Bronx Hospital and Columbia University.* Evidence of in vivo enamel permeability in a centripetal direction is presented using the following simple experimental arrangement: the cuspid (in situ) of a cat dipped into a solution containing 1 mc. of I^{131} per cubic centimeter so that only about 4 mm. of the tip of the intact enamel cusp is wet by the solution. Significant counts over background are read from a Geiger counter placed over the thyroid gland. This establishes the existence of mechanisms which permit the passage of a tissue fluid solute such as I^{131} through intact enamel, dentin, and into the bloodstream with concentration in the thyroid gland. Radioautographic evidence of I^{131} distribution in the tooth and thyroid gland is used to support these findings. In addition, radioautographs are presented to show the distribution of I^{131} throughout the periodontal membrane as well as alveolar bone and gingiva. By this experimental procedure the only source for this uptake is the I^{131} applied to the external surface of intact enamel, in vivo.

2. The Influence of Drugs on Dentin Permeability After Various Time Intervals as Indicated by P^{32} Penetration. *Melvin H. Amler,** College of Dentistry, New York University.* It would be desirable to decrease the permeability of dentin in order to diminish pulpal reaction to external stimuli and lessen the possibility of bacterial invasion. Previous experiments have shown that certain commonly used drugs have the action of increasing dentin permeability. However, some doubt existed as to what the actual effect of these drugs on dentin permeability would be after extended time intervals. These experiments attempted to measure the effect commonly used medicaments have on the dentin permeability. This was indicated by P^{32} penetration after intervals of 7, 14, and 70 days that the medicament was actually in contact with the dentin surface. The results are as follows. Two per cent fluorine caused an increased penetration of P^{32} but after 14 days a decrease in the permeability of the dentin was noted. Seventy per cent ethyl alcohol produced a slight but constant increase in permeability. Silver nitrate followed by eugenol, phenol, and cavity varnish produced an increase in the dentin permeability in proportion to the length of time they were in contact with the dentin. Zn oxyphosphate cement appears to have blocked the P^{32} penetration mechanically, but, when removed after 70 days, caused an increased permeability similar to the other drugs.

3. Introduction to X-ray Diffraction. *Edward Klein, College of Dentistry, New York University.* Explanation of x-ray diffraction phenomena and of some crystallographic terminology will be presented together with a description of various x-ray diffraction techniques.

4. X-ray Diffraction Study of Dental Enamel. *Otto R. Trautz, College of Dentistry, New York University.* A microcamera for the study of tooth sections will be described. Advantages and limitations of this method of investigation and interpretation of the diffractograms will be discussed.

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*Harry H. Shapiro, presiding.

**Guest.

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